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IMPACTS OF DISTRICT METERED AREAS IMPLEMENTATION ON WATER QUALITY IN A FULL-SCALE DRINKING WATER DISTRIBUTION SYSTEMS

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présentée par : DIAS Vanessa Cristina

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DEDICATION

In memory of my beloved father

who is always with me inside my heart.

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RÉSUMÉ

La mise en place de zones sectorisées associée à la gestion de la pression est une approche efficace pour réduire le débit des fuites ainsi que le nombre de bris dans les systèmes d'eau potable. Entre autres, cette stratégie permet de prolonger la durée de vie des conduites et de reporter le renouvellement du système. Toutefois, la création de zones sectorisées implique la fermeture de vannes entraînant la création de nombreux culs-de-sac, qui sont des sites connus pour potentiellement présenter des temps de séjour plus élevés et de l'eau stagnante. De plus, la gestion de la pression produit des vitesses de l'eau plus basses, l'augmentation du temps de séjour et, par conséquent, l'augmentation du risque de la détérioration de la qualité de l'eau. Malgré les succès des zones sectorisées dans un certain nombre de cas rapportés par les municipalités, jusqu'à présent, l'impact de cette stratégie sur la qualité de l'eau n'a pas été entièrement quantifié. En fait, les résultats des études terrain publiées concernant l'impact sur la qualité de l'eau sont très limités. Comme la mise en place des zones sectorisées est certainement une approche intéressante à prendre en compte pour les services de l'eau potable, il nous paraît important d'évaluer l'impact de la mise en place des zones sectorisées sur la qualité de l'eau en utilisant un plan d'échantillonnage détaillé qui tient compte des changements des opérations du réseau ainsi que des variations de la qualité de l'eau à l'entrée du secteur dans cinq zones sectorisées à pleine échelle.

Les objectifs principaux de cette thèse sont de mesurer l'impact de la mise en place des zones sectorisées sur la qualité de l'eau dans un système de distribution d'eau potable à pleine échelle et de développer une stratégie visant à limiter les campagnes d'échantillonnage dans la zone sectorisée. De manière plus détaillée, ce projet vise à : (1) évaluer les changements hydrauliques sur des paramètres tels que le nombre de culs-de-sac, les temps de séjour, les directions d'écoulement et les vitesses de l'eau pendant la mise en place des zones sectorisées; (2) évaluer les changements de la qualité de l'eau avant et après la mise en place des zones sectorisées sur différents types de sites d'échantillonnage à l'intérieur et à l'extérieur des frontières des zones sectorisées; (3) vérifier si les sites de surveillance de la conformité de la qualité de l'eau, utilisés par la municipalité, permettent de détecter des changements de la qualité de l'eau occasionnés par la mise en place des zones sectorisées; (4) développer et valider une procédure mathématique pour prédire les concentrations de trihalométhanes dans la zone sectorisée afin de limiter les campagnes d'échantillonnage; (5) déterminer les changements des communautés bactériennes lors de la mise en place des zones sectorisées et les associations avec d'autres paramètres de qualité de l'eau; et

(6) évaluer si les communautés bactériennes détectées dans l'eau traitée déterminent les structures des communautés bactériennes dans le réseau d'eau potable, ainsi que dans les conduites d'un grand bâtiment, comprenant des données avant et après la mise en place des zones sectorisées.

La première étape était de quantifier l'impact de la mise en place des zones sectorisées sur les conditions hydrauliques ainsi que sur plusieurs paramètres de la qualité de l'eau. Les enquêtes préliminaires ont été effectuées en utilisant des modèles hydrauliques calibrés pour évaluer l'impact de la mise en place des secteurs sur les paramètres qui peuvent influencer la qualité de l'eau (temps de séjour de l'eau, vitesses de l'eau et changements sur le sens d'écoulement) dans le système et pour guider la sélection des sites d'échantillonnage. Une fois ces aspects établis, les sites d'échantillonnage ont été sélectionnés et attribués à différents types de sites à l'intérieur et à l'extérieur des frontières des secteurs. Sur le terrain, des évaluations approfondies et détaillées de plusieurs paramètres de qualité de l'eau (pH, température, turbidité, chlore résiduel, sous-produits de désinfection, métaux, etc.) ainsi que de l'abondance des bactéries (bactéries hétérotrophes aérobies et bactéries totales) ont été menées dans les différents sites d'échantillonnage avant et après la fermeture de vannes. Les résultats des simulations hydrauliques ont montré des changements mineurs du temps de séjour global de l'eau, mais une augmentation significative (10-40%) des sites avec un temps de séjour élevé (> 50h) dans 4/5 secteurs. Les vitesses de l'eau ont présenté de variations mineures. Dans l'ensemble, les changements hydrauliques occasionnés par la fermeture de vannes ont été minimes à modérés. Le chlore résiduel, la turbidité, le fer et le manganèse sont les paramètres les plus influencés par la fermeture des vannes à des endroits tels que les culs-de-sac, les sites à l'extérieur des secteurs et les extrémités. En outre, certains culs-de-sac créés ont montré une qualité de l'eau similaire ou pire que les culs-de-sac existants qui ont été surveillés. Les paramètres qui ont été clairement influencés par les tendances saisonnières, par les variations observées dans les usines de traitement et/ou par le système de distribution lui-même en amont des zones étudiées sont la température, le pH, le carbone organique dissous, le chlore résiduel, les sous-produits de désinfection et les bactéries totales. À partir de la surveillance intensive sur le terrain, un modèle prédictif a été construit et validé au moyen d'une régression linéaire multiple visant à prédire les concentrations de trihalométhanes dans les zones sectorisées à l'aide des paramètres mesurés à un site d'entrée et de la modélisation hydraulique afin d'optimiser la surveillance future dans ce système. L'impact du temps de séjour de l'eau sur l'abondance bactérienne a été observé dans les culs-de-sac créés, les sites à l'extérieur du secteur, et les

extrémités tandis que pour la plupart des culs-de-sac existants, ces concentrations bactériennes n'ont pas varié. L'investigation sur l'abondance bactérienne a clairement montré que les culs-de-sac créés sont des sites critiques pour les bactéries hétérotrophes aérobies, et, dans une moindre mesure, pour les bactéries totales.

Des études sur les structures communautaires bactériennes planctoniques ont été menées avant et après la mise en place des secteurs sur trois sites d'échantillonnage (entrées, culs-de-sac existants et culs-de-sac créés). La réponse des structures communautaires bactériennes à la mise en place des secteurs a été fonction des caractéristiques hydrauliques et physicochimiques de chaque secteur. Des facteurs affectant la culturabilité (chlore résiduel, carbone organique dissous, métaux, etc.) ont été déterminants dans les changements des compositions taxonomiques. Une investigation plus poussée des structures communautaires bactériennes a été menée pour comprendre si l'eau traitée a joué un rôle déterminant dans les communautés détectées tout au long du système de distribution (comprenant des échantillons composites de chaque secteur avant et après la mise en place des zones sectorisées) ainsi que l'influence des conditions de stagnation dans les conduites d'un grand bâtiment. Les résultats de cette étude ont montré des différences significatives sur les communautés bactériennes présentes dans l'eau traitée, l'eau du système de distribution et l'eau du grand bâtiment. Ces résultats confirment que les conditions environnementales spécifiques de chaque environnement, telles que le niveau de l'abondance des bactéries, les concentrations du désinfectant résiduel, les matériaux des conduites, le temps de séjour de l'eau et l'interface biofilm/eau sont des facteurs qui favorisent ou entravent le développement de microorganismes spécifiques.

Nos résultats montrent l'importance de réduire au minimum, autant que possible, le nombre de sites avec temps de séjour élevé lors de la conception de zones sectorisées. La propension des zones sectorisées à influencer les évènements de décoloration a été observée. La surveillance des secteurs doit tenir compte de la qualité de l'eau entrante et les sites d'échantillonnage doivent être choisis avec soin pour éviter une fausse perception de l'impact de la mise en place des zones sectorisées. Toutefois, des recherches supplémentaires sont nécessaires pour vérifier l'influence de la gestion de la pression et des conditions de la demande sur la qualité de l'eau ainsi que l'évolution des communautés bactériennes dans les culs-de-sac et son importance sanitaire.

Enfin, les travaux réalisés au cours de ce doctorat contribuent de façon originale à la définition d'une méthode d'échantillonnage permettant de faire une distinction entre l'effet de la mise en place des zones sectorisées et les effets des changements ou des conditions de qualité de l'eau saisonnière dans le système en amont de la zone de surveillance. En outre, nous avons procédé à une investigation des facteurs de qualité de l'eau et des changements hydrauliques influençant la dynamique bactérienne et à l'application d'une combinaison de méthodes pour établir la diversité bactérienne de l'usine à l'eau du robinet. En conclusion, cette thèse démontre l'absence d'un impact important sur la qualité de l'eau suite à la mise en place des zones sectorisées.

ABSTRACT

The implementation of district metered areas (DMAs) associated with pressure management is an efficient approach for water utilities to reduce flow rates of leakage and the number of breaks in drinking water distribution systems. Additionally, this strategy can extend the life of pipes and postpone system renewal. However, the creation of DMAs involves closing boundary valves, which form more dead-ends in the system. Dead-ends are locations known to potentially present higher water residence times and stagnant water. Additionally, pressure management results in lower water velocities increasing residence times and consequently the potential for water quality deterioration. Despite a number of DMAs being successfully reported by water utilities, until now, the impact of this strategy on water quality has not been thoroughly quantified. Indeed, published field studies regarding the impact on water quality have very limited results. Since DMAs implementation is certainly an interesting approach for water utilities to consider, the work outlined in this thesis focused in better understand their impact on water quality throughout detailed surveys in five pilot DMAs in a full-scale distribution system.

The main objectives of this thesis are to measure the impact of DMAs implementation on water quality in a full-scale drinking water distribution system and to develop a strategy to determine adequate sampling in DMA areas. On a more detailed level, the six specific objectives of this project are: (1) to evaluate hydraulic changes in parameters such as the number of dead-ends, water residence times, flow directions, and velocities during DMAs implementation; (2) to assess the differences in water quality before and after DMAs implementation in different types of sites inside and outside the boundaries; (3) to verify whether compliance monitoring sites allow for the detection of water quality changes; (4) to develop and validate a mathematical procedure to predict concentrations of trihalomethanes in DMAs to limit sampling campaigns efforts; (5) to determine changes in bacterial communities after DMAs implementation and associations with other water quality parameters; and (6) to assess whether bacterial communities detected in treated water determine community structures in the distribution system and premise plumbing of a large building, including data from before and after DMAs implementation.

The first step was to quantify the impact of DMAs implementation on hydraulic conditions and several water quality parameters. Preliminary investigations were conducted using calibrated hydraulic models to assess the impact of DMAs implementation on parameters that can influence

water quality (water residence time, water velocities, and changes in flow direction) in the system and to guide the selection of sampling sites. Once these aspects were established, sample locations were selected and assigned to different types of sites inside and outside DMA boundaries. Extensive and detailed field evaluations on several water quality parameters (pH, temperature, turbidity, chlorine residual, disinfection by-products, metals, etc.) as well as bacterial abundance (heterotrophic plate counts, total bacterial cell counts) were investigated in the different sampling locations before and after DMAs implementation. Results from hydraulic simulations showed minor changes of overall water residence time, but a significant increase (10-40%) of locations with high water retention time (>50h) in 4/5 DMAs. Water velocities presented minor variations. Overall, the hydraulic changes caused by closing valves during the temporary implementation of DMAs were minimal to moderate and their extent depend on the configuration of the DMA. Regarding water quality results from the extensive monitoring, disinfectant residuals, turbidity, iron and manganese were most influenced by the closing of valves at locations such as created dead-ends, sites outside DMAs boundaries, and extremities. Moreover, some newly created dead-ends showed water quality similar or worsen than the existing monitored dead-ends. Parameters that were clearly influenced by seasonal patterns, variations at treatment plants, and/or by the system itself upstream the studied areas were temperature, pH, dissolved organic carbon, chlorine residual, disinfection by-products, and total bacterial cell counts. From the intensive field monitoring, a predictive model was constructed and validated using multiple linear regression to predict trihalomethanes concentrations in DMAs area using parameters measured at an inlet site and hydraulic modeling in order to optimize future monitoring in this system. The impact of water residence time on bacterial abundance was observed in created dead-ends, outside sites, and extremities while existing dead-ends mostly remained with constant levels. Bacterial abundance investigations clearly showed that dead-ends were critical sites for heterotrophic plate counts, and in a minor level for total bacterial cell counts.

Investigation on the planktonic bacterial community structures was conducted before and after DMAs implementation at three sampling sites (inlets, existing and created dead-ends). The response of bacterial community structures to DMAs implementation by type of sampling site was idiosyncratic, depending on hydraulic and physicochemical features of each DMA. Factors affecting culturability (chlorine residual, DOC, metals, etc.) were determinant in the changes of the taxonomic compositions. Further, a detailed systemic investigation on the planktonic bacterial

community structures was conducted to understand whether treated water was determinant in the communities detected along the system (including pooled data from each DMA before and after implementations), as well as the influence of stagnation conditions in premise plumbing. Findings from this investigation show significant differences on the bacterial communities present in the treated plant effluent, water in the distribution system, and water from premise plumbing. These results support that specific environmental conditions such as the level of disinfectant residual, bacterial abundance, pipe material, water residence time, and biofilm/water interface of each sub-system are factors that promote or inhibit the development of specific microorganisms.

Our results show the importance of minimizing, as much as possible, the number of sites with anticipated elevated water residence time when designing DMAs. A propensity of DMAs to influence discoloration events was observed. Monitoring of DMAs should take into account the incoming water quality and sampling sites must be carefully chosen to avoid a false perception of DMAs impact. However, additional research is required to verify the influence of pressure management and the demand conditions on the water quality, as well as the evolution of microbial quality in dead-ends and its sanitary significance.

Finally, this thesis provides original information in the definition of a sampling approach to distinguish the effect of DMAs implementation from those affected by seasonal water quality changes or conditions in the system upstream the area of monitoring. Furthermore, we provide an investigation of water quality factors and hydraulic changes influencing bacterial dynamics. Also, we have applied a combination of methods to establish the bacterial diversity from the plant to the tap. In conclusion, this thesis demonstrates the absence of an important water quality impact following DMAs implementation.

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LIST OF SYMBOLS AND ABBREVIATIONS

ASCE	American Society of Civil Engineers
APHA	American Public Health Association
AWWA	American Water Works Association
Cl_2	Chlorine
CFU	Colony forming unit
Cu	Copper ions
DBPs	Disinfection by-products
DMA	District metered area
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DS	Distribution system
DWDS	Drinking water distribution system
EPA	Environmental Protection Agency
Fe	Iron
HAAs	Haloacetic acids
HPCs	Heterotrophic plate counts
HTS	High-throughput sequencing
in	inches
IWA	International American Water Association
L	Liters
Km	Kilometers
n	Number of observations

m^3	Cubic meter
mg	Milligrams
mgd	Millions of gallons per day
min	Minutes
Mn	Manganese
NRW	Nonrevenue water
NSERC	Natural Sciences and Engineering Research Council
OMBI	Ontario Municipal Benchmarking Initiative
OPs	Opportunistic pathogens
OPPPs	Opportunistic premise plumbing pathogens
PVC	polyvinyl chloride
rRNA	Ribosomal ribonucleic acid
spp.	Species
THMs	Trihalomethanes
UKWIR	UK Water Industry Research
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
WRF	Water Research Foundation
WRT	Water residence time
µg	Micrograms

LIST OF APPENDICES

CHAPTER 1 INTRODUCTION

1.1 Background

Large amounts of treated drinking water carried by distribution systems (15 to 50%) are lost before reaching the customer, not generating any revenue for water utilities (American Water Works Association (AWWA), 2003; Jakubic, 2007; Magini *et al.*, 2007; Smeets *et al.*, 2009). Losses of treated and pressurized water, known as real losses, are comprised of breaks and leaks from water mains and customer service connection pipes, joints, and fittings; from leaking reservoir or tanks walls; and from reservoir or tank overflows (American Water Works Association (AWWA), 2009). The level of these losses depends on the quantity of leaks occurring, their magnitude, the operating pressure, and the duration that the leakage occurs. Furthermore, the volume of losses from many small leaks can surpass that from large reported breaks, since the latter have a run time often limited to a period of hours while small and hidden leaks may last undetected for months or even years (Fanner *et al.*, 2007; Thornton *et al.*, 2008). The World Bank (Kingdom *et al.*, 2006) estimates almost 33 billion m^3/year of real water losses worldwide. In addition to being a waste of resources (water and energy), leakage can be a risk to public health caused by external contaminants entering the pipe through leak openings (American Water Works Association (AWWA), 2009).

Maintaining the integrity of drinking water distribution systems (DWDSs) is essential since this is the final barrier before delivery of drinking water to consumers. The principal control tools for breaks and leaks are pressure management and infrastructure replacement (Thornton, *et al.*, 2008). In North America, aging infrastructure is near the end of its useful life (American Society of Civil Engineers (ASCE), 2009) and has been the leading cause of failures and high leakage rates compromising DWDSs efficiency (Ontario Municipal Benchmarking Initiative (OMBI), 2008; American Water Works Association (AWWA), 2009). Nevertheless, since renewal is a very expensive long-term solution, pressure management is a cost-effective way to prevent new leaks in existing aging pipe systems, along active leakage control as well as optimized repairs to extend the life of pipes (Fanner, *et al.*, 2007). Moreover, pressure management reduces flow rates from leakage and new break frequencies and has been successfully used in combination with district metered areas (DMAs) to reduce and identify leaks in DWDSs (Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009; Kunkel & Sturm, 2011).

DMAs consist in dividing the system into discrete areas, typically from 500 to 3,000 service connections. In this way it is possible to measure flow rates and quantify leakages. However, this practice involves the closing of boundary valves creating dead-ends and hydraulic changes that may result in stagnation, increased water residence time (WRT), and therefore water quality degradation. Factors that can be affected by water residence time and stagnation include several chemical, biological, physical and aesthetic issues such as loss of disinfectant residual (Desjardins *et al.*, 1997; Prévost *et al.*, 1997; Prévost *et al.*, 1998; Baribeau *et al.*, 2001; Rodriguez & Sérodes, 2001; Simard *et al.*, 2011; Machell & Boxall, 2014), disinfection by-products (DBPs) formation (Desjardins, *et al.*, 1997; Speight & Singer, 2005; Rodriguez *et al.*, 2007; Mouly *et al.*, 2010; Simard, *et al.*, 2011), microbial regrowth (Maul *et al.*, 1985; Desjardins, *et al.*, 1997; Prévost, *et al.*, 1997; Prévost, *et al.*, 1998; Carter *et al.*, 2000; Zhang & DiGiano, 2002; Baribeau *et al.*, 2005b; Chauret *et al.*, 2005; Machell & Boxall, 2014), sediment deposition (Gauthier *et al.*, 1999; Barbeau *et al.*, 2005; Vreeburg & Boxall, 2007), corrosion (Eisnor & Gagnon, 2004; Nawrocki *et al.*, 2010), discoloration (Clement *et al.*, 2002; Imran *et al.*, 2005; Vreeburg & Boxall, 2007), and taste and odor problems (Khiari *et al.*, 2002; Maillet *et al.*, 2009).

Despite a number of DMAs implementation being successfully reported by water utilities (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; Kunkel & Sturm, 2011), the published literature presents limited results regarding DMAs impact on water quality from fieldwork studies. Indeed, the limited studies available are either conducted after the implementation, or only once before and after the implementation. As DMA implementation may cause significant changes in the hydraulic parameters that drive water quality, it appeared important to assess the impact of DMA setups on water quality using a comprehensive sampling plan that accounts for changes in distribution operations (WRTs, flow velocities, pressures, etc.) and seasonal variations of incoming water quality (treatment changes, pH, temperature, chlorine residuals, organic matter, metals, bacterial loads, etc.). It also appeared important to expand the water quality parameters considered to provide a better understanding of the source and sanitary significance of any water quality changes.

Consequently, since DMAs implementation is certainly an interesting approach for water utilities to consider, the work outlined in this thesis focused on quantifying the impact of DMAs implementation on water quality by means of a detailed multi-parameter survey on different types of sampling sites during the temporary setup of five pilots sectors in a full-scale DWDS. Such

detailed monitoring constitutes a very significant monitoring commitment and is not feasible outside of a research project scope. In order to assist utilities, we have also developed a sampling approach that can be used combining limited field sampling, hydraulic modeling, and water quality modeling to predict changes in regulatory compliance for DBPs.

1.2 Structure of dissertation

The remainder of this thesis is organized in the following seven chapters. A review on water losses in DWDS, DMAs application and water quality investigations as well as the impact of increasing WRT and hydraulic changes on water quality degradation is provided in Chapter 2. Then, the research objectives, hypotheses and methodology are presented in Chapter 3. Chapters 4 through 6 present our research results in the form of three submitted scientific publications. The first article presents findings from the investigation of the impacts of DMAs implementation on water quality as well as an approach to predict trihalomethanes (THMs) concentrations in DMAs area using parameters measured at an inlet site (Chapter 4, accepted with revisions by Journal AWWA). The following chapter presents findings concerning bacterial changes, including community structures, during DMAs application and association with other water quality parameters (Chapter 5, submitted to Plos One). Chapter 6 reports the results of factors affecting bacterial abundance and community structures along DWDS from treated water to premise plumbing (submitted to Applied and Environmental Microbiology). Finally, a general discussion is provided in Chapter 7 followed by conclusions and recommendations in Chapter 8.

CHAPTER 2 CRITICAL REVIEW OF THE LITERATURE

The purpose of this chapter is to provide a better understanding about real water losses occurring in drinking water distribution systems (DWDSs), the importance and benefits of reducing it as well as presenting the most effective tool commonly used to manage it. This strategy is pressure management associated with the implementation of district metered areas (DMAs). Despite its many benefits, this strategy could increase water residence time (WRT) and the number of stagnant sites in water networks. The published literature is scarce about the impact of increased WRT and the number of dead-ends after DMA implementation. Few studies have been conducted on water quality and can actually quantify the impact of that implementation. They are presented as well as some modeling and historical data analysis studies. Then, the last section presents the potential changes occurring in water quality with time across DWDS.

2.1 Water loss in DWDS

Water loss occurring within DWDS corresponds to the volume of water produced in treatment plants that does not reach the customer, thus not generating revenue for water utilities (American Water Works Association (AWWA), 2009). These losses can be real or apparent losses. Real losses are the physical losses of water comprised of breaks and leaks from water mains and customer service connection pipes, joints, and fittings; from leaking reservoir or tanks walls; and from reservoir or tank overflows. On the other hand, apparent losses are the nonphysical losses that occur when water is delivered to the customer but is not measured or recorded accurately. According to the International Water Association (IWA) and the American Water Works Association (AWWA) water balance methodology, the sum of real and apparent losses plus unbilled authorized consumption is known as nonrevenue water (NRW). The World Bank (Kingdom, *et al.*, 2006) estimates that the worldwide NRW volume amounts to 48.6 billion m^3/year (from an annual production of 300 billion m^3/year), and from this amount almost 33 billion is due to real losses. In Toronto (Canada), a conservative estimate of 25% represents a loss of more than 120 million m^3/year, which could fill more than 50,000 Olympic swimming pools (Jakubic, 2007). In the United States, most states have policies and regulations that limit the maximum acceptable value for water loss within the range of 10% to 15% (United States Environmental Protection Agency (USEPA), 2010). Nevertheless, water loss percentages in the United States can reach more than

30% in some distribution systems (American Water Works Association (AWWA), 2003). The following subsections will focus on the real losses in distribution systems occurred largely due to leakage.

2.1.1 Causes, types and duration of real losses

The most common causes of leakage are pipe age, materials, human error (workmanship defects, poor installation, mishandling of materials prior to installation), environmental conditions (corrosion, weather, vibration and traffic loading) and operational (incorrect backfill, pressure transients, operating pressure, lack of proper scheduled maintenance) (Thornton, *et al.*, 2008). In North America, aging infrastructure is near the end of its useful life (American Society of Civil Engineers (ASCE), 2009) and has been the leading cause of failures compromising system efficiency (Ontario Municipal Benchmarking Initiative (OMBI), 2008; American Water Works Association (AWWA), 2009). The United States utilities needs are estimated at least $11 billion to replace aging facilities that are near the end of their useful life (American Society of Civil Engineers (ASCE), 2009). The cost to renew Ontario's municipal systems was estimated at $34 billion (Jakubic, 2007). In the City of Toronto, for example, half of the water infrastructure is at least 50 years old and almost 10% dates to over a century.

In a study conducted in 21 Canadian cities, cast-iron pipes were the most susceptible to break, followed by ductile-iron, asbestos-cement, and PVC (Rajani & McDonald, 1995). In Montreal, most breaks were also observed in cast-iron and ductile-iron pipes (Besner *et al.*, 2001). Studies in European countries found that leakage is generally low in networks with high proportion of PVC subjected to low water pressures (Smeets, *et al.*, 2009). LeChevallier *et al.* (2006) analyzed historical data of leak and repairs verified that most leaks occurred during the winter months and most repairs were performed on small (eight-in) cast-iron pipes that exhibited a circular type of break associated with pipe movement.

Real losses in pipes can occur as reported, unreported, and background leakage (Thornton, *et al.*, 2008). Figure 2.1 exemplifies the three types and appropriate tools to control them. Reported breaks and leaks are visible and disruptive producing high flow rates being reported to the utility by consumers or utility personnel. Their principal control tools are pressure management and infrastructure replacement. Unreported breaks and leaks are hidden from above ground view producing moderate flow rates being controlled by pressure management and located through

active leak detection. Background leakage corresponds to combined weeps and seeps at joints and on customer service connections presenting typical small flow rates (4 L/min). Also, background leakage is pressure-sensitive and can be addressed by pressure management or infrastructure replacement. However, unlike unreported leakage, background leakage is not detected by conventional acoustic leak detection equipment.

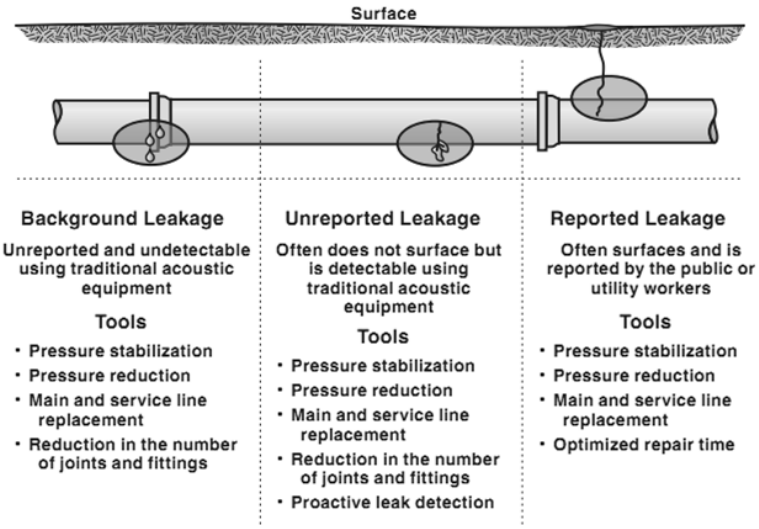

Figure 2.1: Components of leakage and intervention tools (Source: Tardelli Filho (2006))

The level of leakage losses in a distribution system depends on the quantity of leaks occurring, their magnitude, operating pressure, and the total time that the leakage runs (American Water Works Association (AWWA), 2009). Consequently, to contain leakage loss volume it is essential to minimize the duration of individual leaks and to control the operating pressure. The run time of a leak corresponds to three periods: the awareness time (the time it takes for the water utility to become aware that a leak exists), the location time (the time required to locate the leak), and the repair time (the time to conduct repairs of the leak). Furthermore, the volume losses from many small leaks can surpass the volume loss from large reported breaks, since the latter have a run time often limited to a period of hours while small and hidden leaks may go undetected for months or even years (Fanner, *et al.*, 2007; Thornton, *et al.*, 2008).

2.1.2 Reported rates of breaks and leakage losses

Data from water mains breaks in a DWDS shows the occurrence of pipe leaks and are an indicator of infrastructure conditions. A survey conducted by the American Water Works Association (AWWA) (2003) in 337 utilities in the United States and Canada presents an estimate of the mean main break of 13 per 100 Km per year. Another survey including data from 202 utilities from 2003 and 2004, primarily in the United States (2% from Canada), reported higher median values according to utility size: 13.3 breaks per 100 Km per year (<10,000 inhabitants) to 43.8 breaks per 100 Km per year (>500,000 inhabitants) (Lafferty & Lauer, 2005). A similar influence of increased water main breaks as a function of the population size was reported for Quebec cities (Guindon *et al.*, 2008). The break rates per 100 Km per year varied from 7 (municipalities above 3,000 inhabitants) to 44 (municipalities between 50,000 to 100,000 inhabitants). Data from 12 Ontario municipalities show that the city of Toronto, which is the densest city included in that study, has the highest average main break rate: 26 breaks per 100 Km per year (Ontario Municipal Benchmarking Initiative (OMBI), 2008, 2009). However, the city of Windsor with a population of less than 250,000 inhabitants presents the second worst-case value (23 breaks per 100 Km in 2006) among the 12 cities in the report. Average main break rates for the Ontario cities range from 3.9 to 25.8 per 100 Km per year.

The percentage of water loss is widely used to express leakage losses in DWDS. In a typical distribution system 10% of water produced is often lost as leakage (Kirmeyer *et al.*, 2001b) and acceptable values can go up to 15% (American Water Works Association (AWWA), 2003; United States Environmental Protection Agency (USEPA), 2010). However, these losses tend to be larger. A survey conducted by the American Water Works Association (AWWA) (2003) from 35 water utilities in the United States shows loss percentages ranged from 15% to 35%, of which Philadelphia city (the largest population served) showed a loss of 31% and Warren County city (the smallest population served) had a loss of 17%. In Canada, 13 to 40% of treated water is lost before it arrives to the taps in Ontario cities (Jakubic, 2007). In 16 European cities, leakage rates ranged from 3% (in The Netherlands) to 50% (in Bulgaria) (Smeets, *et al.*, 2009). Although the Italian leakage average presented by Smeets, *et al.* (2009) is 28%, in some cities water losses can be more than 50% (Magini, *et al.*, 2007). It should be noted that in the same distribution system, water losses could vary greatly from one sector to another. As discussed above, the age of the system has great influence on water loss. In the Holy city of Makkah (Saudi Arabia), for example,

field investigations in seven areas have shown variations in water loss ranging from 6% to 56% with an average of 32% (Al-Ghamdi & Gutub, 2002). The old areas of this system, built in 1978-79, showed the highest water loss with an average value of 46%. While the newest areas, built between 1990-94 had an average value of 12%.

2.1.3 Importance and benefits of reduce water loss

The integrity of DWDSs is essential since this is the final barrier before delivery of drinking water to consumers. Leakage can lead to customer inconvenience, damage to infrastructure, excessive costs, increased loading on sewers, introduction of air into the distribution network and risk to public health caused by contaminants entering the pipe through leak openings (Farley, 2001; National Research Council of the National Academies, 2006; American Water Works Association (AWWA), 2009). The distribution system has been identified as the most vulnerable part of the multiple barrier system because of the outbreaks caused by accidental intrusion, as well as its potential for deliberate attacks (Lindley & Buchberger, 2002). The failures in the distribution system can have a contribution around 15% of the overall rate of gastroenteritis in the population (Hunter *et al.*, 2005). However, since many outbreaks are not reported, the true impact of distribution systems in waterborne diseases is estimated to be much higher (Craun *et al.*, 2010). The contamination by intrusion occurs when the pressure surrounding the water main exceeds the internal pressure in the pipe, so the water present external to the pipes may flow in through the leak openings. Studies have shown the effects of pressure transients on distribution system water quality (Kirmeyer *et al.*, 2001a; Karim *et al.*, 2003; LeChevallier *et al.*, 2003; Besner *et al.*, 2008; Besner *et al.*, 2010). Sources of contamination that can potentially intrude into the pipe under favorable conditions include soil, non-potable water, groundwater, and sewage. Pathogens and indicators of fecal contamination have been identified in the environment external to water distribution mains (Kirmeyer, *et al.*, 2001a; Karim, *et al.*, 2003; Besner, *et al.*, 2010).

Moreover, since water demand is increasing and resources are diminishing around the world, the application of available intervention tools is essential in order to optimize water losses in DWDS. The management of water losses has many benefits for the utilities, consumers, and for the environment including reduced costs of captation, treatment, operation, and pumping, increase revenues, water conservation, increased level of service to consumers through increased reliability of supply, improving public perception of water companies (Thornton, *et al.*, 2008).

2.2 Pressure management associated to DMAs as a strategic tool to control and minimize water losses in DWDS

Real losses in DWDS can be controlled and assessed by implementing an effective leakage management including four key control activities (American Water Works Association (AWWA), 2009):

1. Pipeline and asset management: pipelines eventually reach the end of their useful life and must be rehabilitated or replaced if they are to continue to provide service,

2. Active leakage control: identifying and qualifying existing leakage in a DWDS, typically by performing leak detection surveys and continuous monitoring of flows into small zones or DMAs,

3. Speed and quality of repairs: repairing leaks in a timely and efficient manner,

4. Pressure management: leakage levels can be improved or worsened solely by changes in the level of operating pressure.

Since system renew is very expensive and a long-term endeavor, pressure management is a cost-effective way to prevent new leaks on existing aging pipe systems. Applying active leakage control as well as optimized repairs will extend the life of pipes (Fanner, *et al.*, 2007). Moreover, pressure management reduces flow rates from leakage and new breaks frequencies and has been successfully used in combination with monitored DMAs to reduce and identify leaks (Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009; Kunkel & Sturm, 2011).

2.2.1 Pressure management

Pressure management in a DWDS involves maintaining pressure at a satisfactory and efficient level avoiding transient pressures and reducing the exceeding pressures, all of which cause unnecessary leaks and breaks (Thornton & Lambert, 2006). This process can be implemented by different tools, including DMAs, pressure sustaining or relief, altitude and level control, transient control and pressure reduction (Fanner, *et al.*, 2007). The benefits of pressure management for leakage control and infrastructure sustainability include the reduction of flow rates from reported and unreported leaks, background leakage, frequency of new breaks, energy costs, inhibition of pressure surges and transients, and extended life of existing infrastructure (Fanner, *et al.*, 2007; American Water

Works Association (AWWA), 2009; Kunkel & Sturm, 2011). Furthermore, the only other way to control background leakage is pipeline rehabilitation/replacement.

Since the rate of leaks or breaks is dependent on the pressure in the system, immediate reduction in the frequency of breaks and leakage has been observed in pressure managed areas in several countries (Lambert, 2000; Thornton & Lambert, 2006). The results after pressure management for 110 sectors from 10 countries shown that the percentage reductions in new breaks (23 to 94%) usually exceeds the percentage reduction in maximum pressure (10 to 75%) (Thornton & Lambert, 2006). The results of DMA implementation and pressure management in the Philadelphia water distribution system showed a significant reduction in leakage and in the 24-hour flow profiles (Kunkel & Sturm, 2011). In this system, leakage has been reduced by 1.19 mgd (from 1.29 to 0.1 mgd) and the 24-hour supply into DMA is about 1.37 mgd less than values before implementation.

2.2.2 District metered areas (DMAs)

DMAs are discrete areas of the distribution system that are sufficiently small (500 – 3,000 customer service connections) to measure and segregate flow rates and quantify leakage events. By limiting supply into the DMA to one or two water mains (Figure 2.2), daily and seasonal variations in flow can be accurately measured by meters placed on the supply mains (Farley & Trow, 2007). The design of a zone for active leakage control has the following aims (Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009): 1) to divide the system into a number of zones with a defined boundary and appropriately sized. In this way, flows can be monitored and unreported leaks can be distinguished from levels of normal consumption by analyzing flow patterns during minimum consumption; 2) to manage pressure in each zone at the optimum level of pressure, consequently inhibiting the increase of new leaks and removing transients that cause breaks.

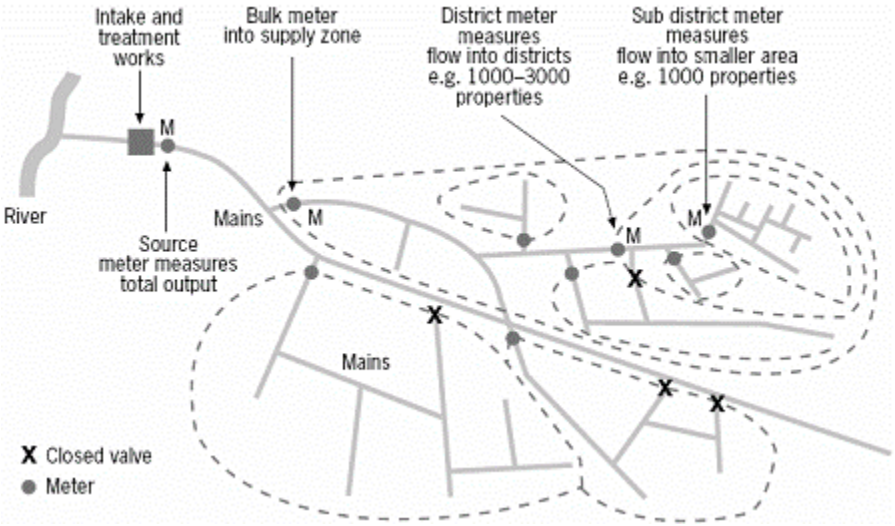

Figure 2.2: Division of a DWDS into DMAs (Source: Farley and Trow (2007))

DMA implementation allied with pressure management is an efficient strategy for water utilities willing to employ active leakage control with more accurate measure of water supplied, enabling the identification and quantification of leaks, reduction of pipe breaks, and extension of pipes service life (Thornton, *et al.*, 2008). Moreover, this strategy helps stabilize the network operation, incidents can be contained more easily and their effect will be on a smaller number of consumers (UK Water Industry Research (UKWIR), 2000). However, creating a DMA involves closing boundary valves which create more dead-ends than would normally be found in the system. These locations are known to potentially present stagnation and higher water retention times (WRTs). Additionally, the association with pressure management results in lower water velocities increasing residence times in the sector and consequently the potential for water quality degradation (Brandt *et al.*, 2004; Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009). Thus, various chemical, biological, physical and aesthetic processes can take place and cause a number of bad side effects in DWDS. These include loss of disinfectant residual, DBPs formation, nitrification, microbial regrowth, sediments deposition, corrosion, discoloration, and taste and odor issues (Smith, 2001; United States Environmental Protection Agency (USEPA), 2002a; Brandt, *et al.*, 2004; National Research Council of the National Academies, 2006). On the other hand, some authors argue that the creation of DMAs allows the water utility to focus more specifically on

valves, fire hydrants, pressure and water quality than in a typical open system (Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009).

2.3 DMAs application and water quality monitoring

This section presents published literature regarding DMAs implementation and water quality monitoring. Despite sectored areas being prone to have high WRT and more dead-ends, systems that applied this practice have reported no significant changes in water quality supported by scarce data (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; Kunkel & Sturm, 2011). The published literature is insufficient and presents limited results regarding DMAs impact on water quality from fieldwork studies. Despite that, historical data have shown that DMAs could have an impact on discoloration events and higher customer complaints (Armand *et al.*, 2015).

2.3.1 Field cases studies

The effect of DMAs on water quality was investigated by the UKWIR in six DMAs two years after their implementation (UK Water Industry Research (UKWIR), 2000). The objective of this field study was to verify whether there was any difference between water quality measured at locations near the natural system dead-ends (existing before closed valves) and that measured near the boundary valves closed during the DMAs implementation. The water quality parameters verified were chlorine concentrations at 18 locations and particulate matter using ALFI filter paper for a monitoring period of 24 hours at two dead-ends (a new and an old one). A network model was used to determine the WRT at the sampling locations. They found similar chlorine concentrations at both location types in the same DMA and minor differences were attributed to differences in WRT. The filter papers did not show any particulate matter in the water during the sampling at both sites. From these results they concluded that no deterioration in water quality occurred as a result of creating DMAs. Nevertheless, they have not verified the conditions in these sites before the DMAs setup and measurements were conducted only once.

In North America, some water utilities have participated in a project using DMAs as an effective strategy to evaluate and reduce water losses (Fanner, *et al.*, 2007). However, among the participants, only Philadelphia created a DMA by isolating an area from the system and could perform an investigation on water quality before and after the DMA creation. Eldorado and Seattle employed existing pressure control zones for the creation of their DMAs and required no changes

in the zones. In Eldorado, no flushing other than the regular hydrant flushing was performed by the utility as well as no water quality problems were reported over the study period. Halifax's fully sub-divided network was divided into permanent DMAs prior to the start of the project, and the main focus was to select a zone with appropriate levels of losses to allow detecting results of pressure control. In Halifax, regular flushing was normally performed to meet water quality standards and during the study period no changes in water quality was observed and no complaints were received from the customers inside the DMA. In the Philadelphia network, water quality monitoring was performed prior to the installation of the DMA and after the DMA was in place for several months. They monitored chlorine residuals inside and outside DMA boundaries at 20 fire hydrants, as well as multi-parameters (e.g., pH, heterotrophic plate counts (HPCs), coliforms, and turbidity) testing at three sites within the DMA (two in the central area and one at a distant location from the main inlet). They verified higher chlorine concentrations at most hydrants (17/20). At the three monitoring locations, lower chlorine concentrations were observed when the DMA was in place, but according to the authors, compatible with the levels found in the system in this period. Also, the furthest site presented levels of turbidity above the accepted range after closing valves. They have concluded that the water quality was comparable within the DMA after closing valves and that acceptable water quality should be maintained in this DMA. However, their conclusions were made based on only one measurement before and one measurement after the isolation. Moreover, the comparison was made six months later (November/December vs. May) during different seasons with probably varying water quality conditions and chlorine dosages.

2.3.2 Hydraulic simulations

The UKWIR also investigated the effect of DMAs on water quality using hydraulic simulations in three models covering rural, sub-urban and urban situations (UK Water Industry Research (UKWIR), 2000). They verified the changes in some water quality parameters (chlorine concentrations, WRT, and sedimentation propensity) before and after the implementations with different scenarios relative to number of DMAs and inlets. They observed minor differences at certain sites between the base model and the DMA model. Near the closed valves, residual concentrations and velocities were lower allowing deposition to occur. Elsewhere in the DMAs, chlorine and velocities increased. The overall conclusion was that the introduction of DMAs had no significant effect on water quality on the parameters monitored. However, they verified that as

the system is divided into more DMAs, more valves are closed and there are consequently more consumers affected by lower velocities or lower chlorine residuals. The criteria used to identify the sedimentation propensity and velocities considered as low were not specified.

Grayman *et al.* (2009) redesigned two existing looped systems in the United States to fit a typical DMA concept and to provide additional control and isolation capability and evaluate the effects in terms of fire flow, WRT, water security and reliability. The first system serves 150,000 people and was divided into 43 DMAs by closing selected pipes with 53 feed lines. The second system serves 100,000 people and was divided into 25 DMAs by closing selected pipes with 27 feed lines. The potential water quality deterioration was verified in terms of WRT, calculated as an average in each system for the last 24-hour period of 192 and 960 hours of hydraulic simulations, respectively. For both systems, no systematic difference in the calculated average WRT between the alternative designs was noticed. However, they observed significant variations in residence time by node, but considered that when viewed as a whole, no important variation was found.

2.3.3 Historical data

The impact of DMAs on increasing the risk of discoloration was investigated by analysing historic data from discoloration customer contacts over a period of six years in a United Kingdom water company (Armand, *et al.*, 2015). The case study area consists of 22 DMAs, 345 Km total length, serving nearly 42,000 people. Discoloration events were grouped in trunk mains (upstream issues), flushing, pipe bursts, and the remaining events are subdivided in four categories according to number of customer contacts (single or multiple) and affected locations (single, adjacent consecutive or scattered pipes within the system). DMAs influence on the discoloration customer contacts was also verified by means of hydraulic simulations comparing the original DMA-based system and a non-DMA-based system (all boundary valves open). The results confirmed that DMAs could have a negative effect on discoloration. More than a third of locations where discoloration events were detected were attributed to the DMA boundary valves. Discoloration contacts are mostly attributed to the pipes with low flow ($\leq$0.06 m/s) and high WRT (linear increase). These conditions were predominant at dead-end locations.

Prasad and Danso-Amoako (2014) analyzed six-years of water quality data from 176 DMAs from a United Kingdom water company covering 36 water quality parameters and customer complaints in order to detect parameters that most influence iron and manganese accumulation. Only a few

parameters such as those related to bio-chemical oxidation and sorption significantly influenced the accumulation of these metals. In the majority of the DMAs, iron and manganese levels were influenced by alkalinity (pH and corrosion control), turbidity, aluminum (used as coagulant), temperature, trihalomethanes (THMs), and free chlorine residuals. Concentrations of iron and manganese above the maximum concentration level in United Kingdom (200 μg/L and 50 μg/L, respectively) were never present when free chlorine concentrations were superior to 0.8 mg/L. The influence of seasonal variations regarding customer complaints data showed high peaks of customer complaints during warmer months (second and third quarters of the year) attributed to increased water consumption with higher velocities releasing accumulated particles and higher temperatures promoting bacterial and chemical oxidation of iron and manganese. A comparative assessment of the water quality prior to DMAs installation was not an objective of this study, however these results show the propensity for water quality issues in this design. Discoloration problems are common in DMAs, and according to the authors, DMAs with high populations have a high propensity to present more customer complaints.

2.3.4 Main findings from DMA surveys

The published studies regarding the impact on water quality associated with DMAs implementation in DWDS have not reported any significant effects caused by the physical and hydraulic changes necessary to the design of this intervention. They have concluded that DMAs do not seem to have long-term effects on water quality or that the creation of DMAs causes both negative and positive impacts but with overall similar or better quality than before, compensating the negative impacts. However, the lack of observed impact on water quality may result from few results available and limited sampling campaigns. Furthermore, systems that have DMAs may experiment more problems related to consumer complaints, such as discoloration, and the need to increased flushing frequency at problematic locations. In the following section, problems related to higher WRTs and stagnation in DWDS are discussed.

2.4 Water quality issues associated with increased water residence time in DWDS

As water flows through the networks, reactions occur in the bulk water as well as with the pipe walls producing several chemical, physical, and aesthetic transformations. WRT has a major effect

on water quality, and the deterioration level within the system will be dependent on the water flow, finished water, pipe materials and deposited materials (United States Environmental Protection Agency (USEPA), 2002a). Water quality problems that can be caused or worsened by time in the distribution system include loss of disinfectant residuals, DBPs formation, microbial regrowth, corrosion, sediments deposition, discoloration, and taste and odor problems (Smith, 2001; United States Environmental Protection Agency (USEPA), 2002a; Brandt, *et al.*, 2004; National Research Council of the National Academies, 2006). Table 2.1 lists these problems, their potential causes, and the parameters that could be monitored in DWDS in order to identify the changes. Some of these issues such as DBPs and microbial regrowth are related to potential public health impacts (United States Environmental Protection Agency (USEPA), 2002b; Richardson *et al.*, 2007; Villanueva *et al.*, 2007). Other issues can be identified by consumers and be a cause of customer complaints such as discoloration, poor taste, and odor impacting user trust in the water utility services (United States Environmental Protection Agency (USEPA), 2002a; Brandt, *et al.*, 2004).

The use of a disinfectant residual is a common practice worldwide for the maintenance of water quality limiting microbial regrowth and protect it from potential contamination across DWDSs (Haas, 1999; LeChevallier, 1999). The consumption of the disinfectant normally occurs with time due to interactions with substances in bulk water, biofilm, and pipe material. These phenomena influence the maintenance of a residual from treatment plants to the extremities within the system while avoiding higher concentrations at treated water or applying rechlorination (Prévost, *et al.*, 1998; Prévost *et al.*, 2014). Higher dosages at plants and/or rechlorination could enhance taste and odor issues as well as additional DBPs formation, mainly in warmer months as the reactions proceed faster at higher temperatures (United States Environmental Protection Agency (USEPA), 2002a; Rodriguez, *et al.*, 2007). In Quebec, the water quality regulation requires at least 0.3 mg/L of free chlorine (or an equivalent of another disinfectant) after treatment or a disinfectant residual that achieves the pathogen removal efficiencies required by the regulation, but no minimum requirement elsewhere in the system is required (Ministère du développement durable de l'environnement et lutte contre les changements climatiques (MDDELCC), 2016). Locations with higher WRTs and stagnation such as dead-ends and extremities are known to be the most susceptible sites to present low or undetectable disinfectant residuals. The effect of increased WRTs, distance from treatment plant, or stagnant locations in relation to the loss of disinfectant residuals in DWDSs have been observed in several studies (Desjardins, *et al.*, 1997; Prévost, *et al.*,

1997; Prévost, *et al.*, 1998; Baribeau, *et al.*, 2001; Rodriguez & Sérodes, 2001; Barbeau, *et al.*, 2005; Simard, *et al.*, 2011; Machell & Boxall, 2014).

Table 2.1: Water quality issues associated with increased residence time (Source: Brandt, *et al.* (2004))

Issues	**Potential causes**	**Parameters of investigation**
Loss of disinfectant residual	- Long WRT - High levels of organic matter in treated water - Unlined cast-iron pipes - Sediments	- Free chlorine - Total chlorine - Chloramines
DBPs	- Long WRT - Inadequate pH control - Poor quality source/water treatment	- THMs - HAAs
Microbial regrowth	- Long WRT - Poor quality source/water treatment - Intrusion - Unlined cast-iron pipes	- Total and fecal coliforms - HPCs
Corrosion	- Inadequate chemical treatment - Stagnation - Long WRT may reduce pH which could increase corrosion	- Lead - Copper - Iron
Discoloration	Red Water - Long WRT in unlined cast-iron - Low alkalinity - Change in flow regime Sediment - Slow velocities resulting in sedimentation of particles - Increase velocities releasing particles to bulk water	- Iron - Manganese - Turbidity - Color - Consumer complaints
Taste and odor	- Chlorine residual - Microbiological activity - Leaching from materials - Phenolic compounds	- Consumer complaints

DBPs are a concern in drinking waters since their exposure is associated with some types of cancer in humans (Sadiq & Rodriguez, 2004; Richardson, *et al.*, 2007; Villanueva, *et al.*, 2007). Factors influencing the formation and levels of DBPs comprise water quality parameters such as pH, temperature, reactivity of organic matter, treatment, chlorination conditions, reaction time, and seasonal variations (Liang & Singer, 2003; Sadiq & Rodriguez, 2004; Brown *et al.*, 2011). THMs and halogenated acetic acids (HAAs) are the two groups of chlorinated DBPs most often regulated under various international legislations (Richardson, *et al.*, 2007). Their levels in DWDSs vary according to water source, treatment, rechlorination, season, and time (Rodriguez, *et al.*, 2007; Simard, *et al.*, 2011). In Quebec, the maximum acceptable level of THMs and HAAs concentrations in DWDS are 80 µg/L and 60 µg/L, respectively (Ministère du développement durable de l'environnement et lutte contre les changements climatiques (MDDELCC), 2016), based on annual averages calculated over trimestral samples. Studies have shown that THM concentrations often increases with WRTs across distribution systems (Desjardins, *et al.*, 1997; Speight & Singer, 2005; Rodriguez, *et al.*, 2007; Mouly, *et al.*, 2010; Simard, *et al.*, 2011). On the other hand, some HAA species have been observed to reach a maximum level and then decline across the system, especially in locations with low disinfectant concentrations and warm temperatures (Rodriguez *et al.*, 2004; Baribeau *et al.*, 2005a; Speight & Singer, 2005). This decrease is attributed mainly to microbial activity, but chemical decomposition can also be a factor.

Microbial regrowth is another concern linked to potential health risks associated with DWDSs that could be worsened by higher WRTs. Microbial regrowth in the water system consists in the growth of bacteria and repair of injured bacteria during the distribution after the treatment. This growth is normally limited by applying disinfectant residuals in the finished water. However, bacterial regrowth and proliferation of resistant pathogens can occur in water networks even in the presence of a residual disinfectant (Prévost, *et al.*, 1998; Zhang & DiGiano, 2002; Berry *et al.*, 2006; Liu *et al.*, 2013). Regrowth can also be aggravated by elevated WRT since the disinfectant residuals decrease as water passes through the pipes within the systems. Several studies have demonstrated that microbiological water quality tends to worsen during distribution of drinking water (Maul, *et al.*, 1985; Prévost, *et al.*, 1997; Prévost, *et al.*, 1998; Zhang & DiGiano, 2002; Machell & Boxall, 2014). However, a decrease can also be observed at low flow conditions in dead-ends, since the bacteria can be settled out of the bulk water before reaching the taps (Carter, *et al.*, 2000). Other factors also causing changes of microbial levels include temperature, concentration of organic and

inorganic compounds, and pipe materials (Laurent *et al.*, 2005a; Prévost, *et al.*, 2014). In Quebec the monitoring requirements for microbial control in the network include total and fecal coliform bacteria, as well as *E.coli* with minimum monitoring sampling according to population size and at least 50% of the samples collected at the extremities of the network (Ministère du développement durable de l'environnement et lutte contre les changements climatiques (MDDELCC), 2016).

Our understanding of microbial dynamics in distribution systems is rapidly progressing with the recent development of more advanced molecular based analytical techniques, such as high-throughput sequencing (HTS) procedures. These recent developments overcome the limitations of more conventional methods, such as culture-based and of previous molecular methods such as fingerprinting (Forney *et al.*, 2004), and enable a high-resolution characterization of microbial populations in DWDSs. These methods offer a high potential for applications in drinking water quality monitoring, furthering our understanding of distribution pipeline ecology. Indeed, problematic scenarios might be linked to specific bacterial groups or species, which can be used as indicators for future monitoring. Bacterial compositions have been investigated in DWDS using HTS or similar techniques in combination with traditional methods to help better understand bacterial diversity in drinking water (Pinto *et al.*, 2012; Lautenschlager *et al.*, 2013; Liu *et al.*, 2014; Prest *et al.*, 2014; El-Chakhtoura *et al.*, 2015; Roeselers *et al.*, 2015). Studies at full-scale have focused on comparing communities present at various stages in the treatment plant, the treated water and at locations of the distribution system (Pinto, *et al.*, 2012; Lautenschlager, *et al.*, 2013; Liu, *et al.*, 2014; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015). Clear and evident changes in the planktonic bacterial community structures are expected to occur between the water's source and water after subsequent steps of treatment (Pinto, *et al.*, 2012). Nevertheless, after treatment, results from several studies using HTS to examine changes occurring in DWDS suggest that planktonic bacterial communities leaving the plant remain relatively stable throughout distribution (Pinto, *et al.*, 2012; Lautenschlager, *et al.*, 2013; Roeselers, *et al.*, 2015). The impact of residence time on planktonic bacterial communities has been studied in a full-scale unchlorinated DS and few changes were noted at higher residence times (≈ 50 hours) (Lautenschlager, *et al.*, 2013), questioning the impact of water residence time and/or the presence of a disinfectant residual on microbiota.

Water stagnation and WRT could also influence corrosion in DWDS (Eisnor & Gagnon, 2004; Nawrocki, *et al.*, 2010). Other water quality problems related to corrosion include reduced age of

pipes, leaks, disinfectant loss, bacterial regrowth, and discoloration by the release of corrosion by-products (McNeill & Edwards, 2001). WRT could play a role in the effect of corrosion by-products in the form of discoloration as red water (Clement, *et al.*, 2002; Imran, *et al.*, 2005). Discoloration is one of the main customer complaints related to drinking water (Brandt, *et al.*, 2004; Vreeburg & Boxall, 2007). It is associated with the movement of accumulated organic and inorganic particles such as those originated from the source water, treatment process, materials from the distribution system, biological regrowth, chemical reactions or external contamination (Vreeburg & Boxall, 2007). These particles are influenced by the flow conditions in the system. They settle and accumulate in the pipes under low flow or stagnation conditions, and then are released to the bulk water when increases or changes in flow occur. Sediments can also influence disinfectant loss and have a relation with microbial regrowth (Gauthier, *et al.*, 1999; Barbeau, *et al.*, 2005). An investigation regarding the benefits of spot flushing in two dead-ends in a DWDS (Barbeau, *et al.*, 2005) showed that the removal of sediments produced short-term impacts in the aesthetic characteristics of the water (turbidity and iron), but no effect on disinfectant residuals and HPCs. The identified removal sediments were mostly constituted of iron, organic matter, and manganese. Increased concentrations of iron and manganese in drinking water could cause discoloration (Slaats *et al.*, 2003). Iron and manganese are not considered health-based parameters in water quality regulations across Canada. However, epidemiological studies suggested significant neurological effect in children associated with manganese exposure in drinking water (Wasserman *et al.*, 2006; Oulhote *et al.*, 2014). Health Canada (2014) recommends a limit of 300 µg/L for iron and 50 µg/L for manganese, since these metals can lead to aesthetic issues and consequently customer complaints.

DWDS could also provide a favorable environment to potentiate taste and odor issues caused by microorganisms and substances presents in the water and across the system pipelines, and may be perceivable during water consumption (United States Environmental Protection Agency (USEPA), 2002a; Brandt, *et al.*, 2004). Investigations identified WRT as a factor influencing the concentration of taste and odors compounds in DWDSs (Khiari, *et al.*, 2002; Maillet, *et al.*, 2009). However, higher levels of disinfectant residuals near the water treatment plant are more evident to customers (Suffet *et al.*, 1996; Turgeon *et al.*, 2004) and could mask the effect of other tastes and odors compounds (Proulx *et al.*, 2012).

CHAPTER 3 RESEARCH OBJECTIVES, HYPOTHESES AND METHODOLOGY

3.1 Objectives

The main objectives of this thesis are to measure the impact of DMAs implementation on water quality in a full-scale DWDS and to develop a strategy to limit sampling campaigns in the DMA area.

On a more detailed level, the specific objectives are to:

1. Evaluate hydraulic changes in parameters such as the number of dead-ends, water residence times, flow directions, and velocities during DMAs implementation;
2. Assess the differences in water quality before and after DMAs implementation at different types of sites inside and outside the boundaries;
3. Verify whether compliance monitoring sites allow for the detection of water quality changes;
4. Develop and validate a mathematical procedure to predict concentrations of THM4 in DMAs to limit sampling campaigns efforts;
5. Determine changes in bacterial communities after DMAs implementation and associations with other water quality parameters;
6. Assess whether bacterial communities detected in treated water determine community structures in DWDS and premise plumbing of a large building, including data from before and after DMAs implementation.

Achieving these objectives will allow us to answer fundamental questions with regards to changes on water quality related to physical modifications occurring when DMAs are implemented:

- Do hydraulic conditions change significantly after DMAs implementation?
- Which water quality parameters are influenced the most by the physical and hydraulic changes?
- Which locations are more affected by DMAs implementation?
- Does monitoring at compliance monitoring sites allow for the detection of water quality changes?

- Are changes in bacterial communities after DMAs implementations in new stagnant sites comparable to existing ones?
- Does treated water determine bacterial communities in distribution systems and premise plumbing from a large building?
- Which water quality parameters influence the bacterial communities the most?

The project objectives are derived from the following research hypotheses:

Hypotheses concerning the impact of DMA implementation on water quality.

1. The implementation of DMAs does not change the overall distribution of the hydraulic parameters (flow velocity and direction, water residence times, etc.) that impact water quality. *Although changes in the hydraulic parameters are to be expected, changes in some locations will be compensated by opposite changes in other sites*.
2. Water quality changes observed after DMAs implementation are associated to changes in distribution conditions or in water quality entering the DMA. *The few field monitoring studies published in the literature suggest that DMAs implementation does not affect water quality. However, these studies were either restricted to analyze the changes only after the implementations, or to monitoring only once in each condition (before/after) with comparisons made after a long period and at different seasons. Furthermore, limited water quality parameters were considered. A rigorous comparison of the water quality before and after DMA implementation is needed to conclude on the impact of isolating distribution system sectors. Detailed monitoring of water quality before and after implementation is needed to base any conclusion on the impact of DMAs. Monitoring results must then be interpreted considering the seasonal water quality changes of the water entering the sectors and the operational conditions (pH, temperature, demand, etc.).*
3. Water quality changes after DMAs implementation are short-term and located at specific sites. *DMA implementation will cause changes in the hydraulic parameters such as flow velocity, flow direction, and residence time. Transient water quality issues can be anticipated.*
4. Water quality at newly created dead-ends is similar to water quality found at previously existing dead-end points. *Water quality problems are commonly reported at new dead-ends after DMA implementation, and may warrant corrective action (flushing, purge,*

reconfiguration, pipe replacement, etc.). There is no information available to demonstrate if similar water quality changes (loss of disinfectant residual, increase in bacterial indicators, metals, and turbidity) occur. Nor is there information available on the composition of bacterial communities in newly formed and existing dead-ends following the implementation of DMAs. A better understanding of water quality changes and bacterial communities in dead-ends will provide insight in how to manage water quality in these critical points after the implementation of a DMA.

5. There are significant differences on the planktonic bacterial communities present in the treated plant effluent, water in the main pipes, and water from premise plumbing. *Previous studies showed a consistency in planktonic bacterial composition from treated water to locations in DWDS, even in networks using residual disinfectants. There is no information on the consistency throughout the whole system including premise plumbing of large buildings. Our results will help further understand the bacterial quality of the distributed water and enable better management of potential bacterial contaminants in DWDSs and premise plumbing.*

Hypotheses concerning the management of DMA implementation.

6. Compliance monitoring sites are not suited to detect water quality changes following DMA implementation. *Compliance monitoring sites are not always present on critical points or near the boundaries, so they may not be influenced by physical and hydraulic changes. Moreover, as these sites are often located inside public buildings, the water quality at the sampling tap may be impacted by the premise plumbing.*
7. It is possible to optimize sampling campaigns in DMAs using data collected only from inlet sites and to predict regulated water quality parameters such as DBPs (THM4) in the area. *Propose and validate a procedure in order to optimize water quality evaluation sampling campaigns.*

3.2 Methodology

The experimental procedure was conducted in the following sequence:

1. Hydraulic network modeling, selection of sampling sites in the DMAs and definition of sampling plans;

2. Field evaluation of the impact of DMAs implementation on water quality;
3. Identification of planktonic bacterial communities in treated water, within DMAs (before and after) and in premise plumbing by Illumina MiSeq sequencing.

3.2.1 Hydraulic network model

Hydraulic simulations were conducted using calibrated models provided by the water utility to assess the impact of DMAs on parameters that can influence the water quality such as the WRT, water velocity and changes in flow direction to guide the choice of sampling sites. The models were calibrated using flow and pressure data obtained from field measurements on fire hydrants before and after DMAs implementation (details in Appendix 1). Simulations were performed on WaterGEMS V8i software with scenarios before and after DMAs implementation using average daily consumption considering hourly variations patterns. In order to assure stability and repeatability, the simulation was carried out over 15 days using extended period simulation and the values obtained from day 10 at the minimum and maximum demand conditions were considered.

3.2.2 Characteristics of DMAs and choice of sampling sites

In 2012 and 2013, five pilot DMAs were implemented across a full-scale DWDS serving nearly 1.5 million residents across around 4,000 km of pipes in Montreal (Canada). The distributed drinking water is produced by two treatment plants supplied by surface water and the treatment process included flocculation, filtration, ozonation and post-disinfection with chlorine. Figure 3.1 and Table 3.1 presents the characteristics of the DMAs, sampling locations and frequencies. These five sectors were selected by the water utility as the priority areas for pressure management based on historical data of each one: breaks frequency, conditions of pipes (year of installation, materials) and pressure of the area.

The selection of sampling sites was performed to detect changes on water quality by comparing results of hydraulic simulations in order to cover various situations resulting from changes caused by DMAs implementation, based on the characteristics of the DMA, characteristics of the site, and changes on flow direction. Depending on the characteristics and size of DMAs, 10 to 13 sampling points were selected. Each point was sampled weekly, at least three times before and three times after the implementation of DMAs, on the same day of the week and the same time of the day (morning peak) to minimize variations due to customer demand for water.

Sampling locations were assigned to one of seven location groups: inlets (to establish the baseline for water quality), existing and created dead-ends after the closing of the valves, outside DMA (near the boundaries), extremities (sites far away from the inlet), sites with changes in flow direction or water age variation, and compliance monitoring sampling points used by the water utility. Sampled pipes in these sectors are made of cast iron (or ductile iron at few locations on DMAs 1 and 5) with internal pipe diameters varying from 4 to 14 inches.

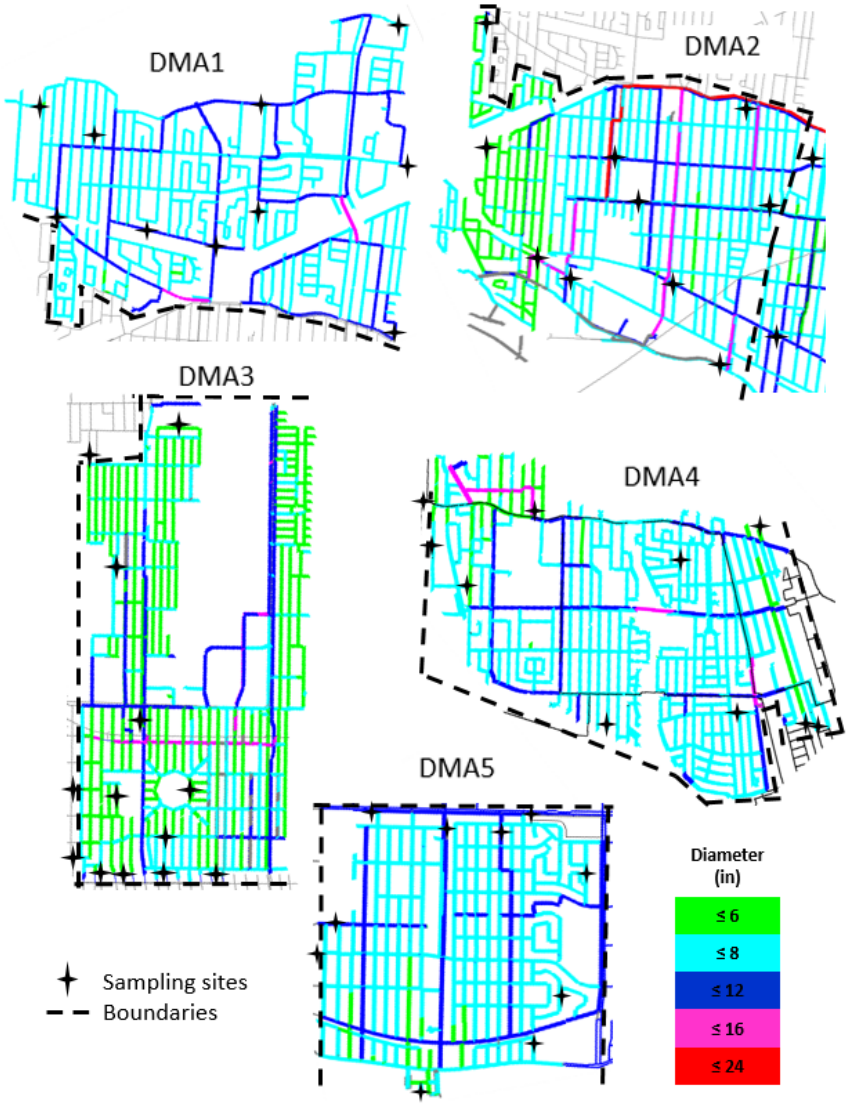

Figure 3.1: DMAs boundaries with pipe diameters and sampling sites locations

Table 3.1: DMAs characteristics and sampling frequency

Characteristics	DMA1	DMA2	DMA3	DMA4	DMA5
Total length (km)	64.3	88.2	99	77.5	52.8
# inlets after implementation	3	4	5	6	4
# closed valves	3	31	19	18	14
Frequency of sampling before	5	3	3	3	3
Frequency of sampling after	5	6	13	3	3
Number of sites sampled	10	12	13	10	10
Sampling dates	06/09-18/10/2012	09/10-22/11/2012	30/05-17/09/2013	09/07-20/08/2013	18/09-29/10/2013

3.2.3 Sampling strategy and water quality analysis

Water quality samples were taken at public buildings (only compliance monitoring sites) and outdoor household taps (both previously disinfected), after refreshing the pipe until the water temperature was constant to confirm that the water from the main pipe had reached the tap. All samples were taken following standard procedures, using sterile bottles, transported to the laboratory at 4°C and processed on the same day.

General water quality parameters, such as temperature, pH, chlorine residuals, turbidity and conductivity were measured at the time of sampling with portable instruments according to standardized methods. Free and total chlorine residual measurements were conducted with the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method using a DR/2010 spectrophotometer (HACH) according to Standard Methods 4500-Cl (American Public Health Association (APHA) *et al.*, 2005). DOC samples were filtered through pre-rinsed 0.45 μm hydrophilic polyethersulfone membranes (Supor®-450, PALL, USA), then analysis measurements were conducted on laboratory total organic carbon analyzer (Sievers Instruments, Inc., GE, USA) according to Standard Methods 5310-C. Disinfection by-product formation was verified by four trihalomethanes (THM4: chloroform, bromoform, bromodichloromethane, dibromochloromethane) and six haloacetic acids (HAA6: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid,

dibromoacetic acid and bromochloroacetic acid). THMs were extracted by liquid/liquid extraction with hexane as described in Standard Methods 6232B (American Public Health Association (APHA), *et al.*, 2005). HAA compounds were extracted by liquid/liquid extraction with methyl tert-butyl ether (MtBE) followed by derivation with acidic methanol in accordance with USEPA Method 552.2 (United States Environmental Protection Agency (USEPA), 1995). All DPBs were analyzed on a gas chromatograph (CP-3800, Varian) equipped with an electron capture detector (GC/ECD) (Column characteristics: capillary column RTX-5MS with 30 m × 0.25 mm ID and 0.25 µm film thickness). Iron and manganese (dissolved and total for both parameters) were analyzed by ICP (Thermo Fisher iCAP 6000). Particle counts were measured with a Brightwell Dynamic Particle analyzer (DPA4100). Total coliforms were measured on m-Endo media after 24 hours at 35°C. Heterotrophic plate counts (HPC) were determined on R2A agar after incubation for seven days at 20°C according to method 9215-D (American Public Health Association (APHA), *et al.*, 2005). Total bacterial cell counts were determined by epifluorescence microscopy (Olympus, Tokyo, Japan) at 1000-fold magnification (Hobbie *et al.*, 1977). Analyses were performed two or three times and averaged for each sample.

3.2.4 Planktonic bacterial communities across DWDS and premise plumbing

This analysis was performed in two phases:

- Phase 1: Processing samples to assess the impact of DMAs implementation on planktonic bacterial communities comparing profiles from inlets, existing dead-ends, and created dead-ends.
- Phase 2: Processing samples to compare the bacterial communities in treated water, distributed water, and premise plumbing water from a large building.

In Phase 1, samples were taken in DMAs 3, 4 and 5 from inlets, existing and created dead-ends before and after DMAs implementation. In Phase 2, a total of 28 water samples were taken from two water treatment plants, five DMAs in the distribution system, and 10 taps in a hospital. Treated water samples were collected four times from each plant after chlorination. The samples were collected at intervals of approximately three weeks between each sampling (during the summer season of 2014). Sampling in the distribution system was performed twice from five sectors (DMA 1 through DMA 5) at intervals of about four weeks. In each sector 10 to 13 samples were collected. DMA 1 and DMA 2 were sampled in the fall of 2012; DMA 3 and DMA 4, in the summer of 2013;

and DMA 5, in the fall of 2013. Samples from premise plumbing (copper material) were taken from standard and pedal taps, from four different floors comprising five hospital departments. Sampling was performed once (summer of 2013) at each of ten taps (TAP 1 through TAP 10) collecting the first cold water flush after one-night of stagnation. All samples were taken using sterile bottles, with sodium thiosulfate (1%) to neutralize the activity of disinfectants. The samples were then transported to the laboratory at 4°C and processed on the same day.

3.2.4.1 DNA extraction and bacterial 16S rRNA gene PCR-amplification and gene sequencing

Samples were filtered through a 0.45 µm pore sized, 47 mm diameter, mixed cellulose ester membrane. Specifically, 10 L of treated water from each plant, and 500 mL of each sample from distribution system and the tap waters were individually filtered. For each DMA, samples were pooled at the time of filtration by mixing equal volumes of water from each sample (500 mL) by type of sampling point and condition (phase 1, totalizing 18 samples) or all sampling points together (phase 2, totalizing 10 samples) corresponding to the same sampling day. All filtration equipment was sterilized by autoclaving prior to filtration. The filter was inserted into an extraction tube containing a garnet matrix and one 1/4-inch ceramic sphere (Lysing Matrix A, MP Biomedicals, Solon, USA) and cut in half. DNA from the obtained biomass was extracted directly on the filters using the bead-beating protocol adapted as previously described (Yu and Mohn, 1999). Lysing buffer was added to each extraction tube prior to the bead-beating step performed on FastPrep-24 (MP Biomedicals), followed by ammonium acetate precipitation, and successive cold 70% ethanol washes. Extracted DNA was stored at -25°C until further analysis performed at Research and Testing Laboratory (Lubbock, TX, USA). The bacterial 16S rRNA gene was PCR-amplified for sequencing using a forward and reverse fusion primer as previously described (MacIntyre et al., 2015). PCR products were sequenced using paired-end Illumina MiSeq sequencing (Illumina, Inc. San Diego, California) 2x300 flow cell at 10pM.

3.2.4.2 Data analysis

Raw sequencing reads were processed with the software Mothur v.1.34.4 (Kozich et al., 2013). Quality filtering of reads was performed to remove low quality and chimeric sequences. Briefly, data were extracted from raw fastq files, and sequences containing ambiguous bases and/or longer than the expected fragment were discarded. Subsequently, sequences were aligned against the Silva

reference alignment (Release 119) (Quast et al., 2013). Potential chimeric sequences were identified using the UCHIME algorithm, and sequences not associated with bacteria were removed. Finally, sequences were clustered into operational taxonomic units (OTUs) with 97% identity. Rare OTUs identified at the 0.005% thresholds were removed (Bokulich et al., 2013). Subsampling was performed to select sequences at random, corresponding to the sampling effort of the smallest library, to avoid downstream bias in statistical analysis due to varying sampling efforts. After quality filtering reads, eliminating rare sequences, and subsampling, the final OTU table was assigned to different taxa levels using the RDP classifier (Release 11) (Wang et al., 2007) with a minimum confidence threshold of 80% for bacteria and 50% for other taxonomic levels. Venn diagrams were created to verify the shared OTUs among sample groups. Mothur was also used to calculate richness estimators and diversity indices. Results of the UPGMA clustering, similarity profile analysis (α=0.05), PCA and indicator species analysis (Indval) were analyzed using vegan, ade4, ape, and indicspecies packages in R software (Paradis et al., 2004; Dray and Dufour, 2007; De Caceres and Legendre, 2009; Oksanen et al., 2015).

Table 3.2 presents a summary of the experimental procedure for each hypothesis together with expected results and the corresponding scientific paper presenting these results.

Table 3.2: Experimental procedure developed to validate (or invalidate) the research hypotheses and corresponding articles

	Hypothesis	Scale	Experimental approach	Expected results	Article
1	The implementation of DMAs does not change the overall distribution of the hydraulic parameters	Hydraulic modeling	Hydraulic modeling of scenarios before and after implementations	Hydraulics parameters before and after implementations Choice of sampling sites	2
2	Water quality changes observed after DMAs implementation are associated to changes in distribution conditions or in water quality entering the DMA	Field: DWDS	Investigation of multi-physicochemical parameters at seven different types of sampling sites in five pilot DMAs	Variations of multi-parameter water quality at inlets before and after DMAs implementation	1-2
3	Water quality changes after DMAs implementation are short-term and located at specific sites	Field: DWDS	Sampling of multi-parameter water quality in five pilot DMAs	Variations of water quality by type of sampling site before and after DMAs implementation	1-2
4	Water quality at newly created dead-ends is similar to water quality found at previously existing dead-end points	Field: DWDS	Apply HTS of 16S rRNA genes to analyze three different sites in a DMA before and after implementation: inlets, created and existing dead-ends	Planktonic bacterial communities at each location Influence of water quality parameters on microbial structures	1-2
5	There are significant differences on the planktonic bacterial communities present in the treated plant effluent, water in the main pipes, and water from premise plumbing	Field: DWDS, premise plumbing (hospital)	Apply HTS of 16S rRNA genes to analyze a full-scale DWDS: treated water, different distribution system sectors, and premise plumbing sites	Planktonic bacterial communities at each location Influence of water quality parameters on bacterial structures and indicators of each location	3
6	Compliance monitoring sites do not allow to identify all the water quality changes	Field: DWDS	Sampling at seven different types of sites in five DMAs	Comparison of results in compliance monitoring sites with the most impacted locations	1-2
7	It is possible to optimize sampling campaigns in DMAs using data collected only from inlets and predicting regulate DBPs such as THM4 in the area	Numerical: multiple stepwise linear regression	All data collected from the first four DMAs were merged together to construct the predictive model and the data from DMA 5 was used for validation	A mathematical model to predict concentrations of THM4 in DMAs using only data collected at inlets	1

CHAPTER 4 ARTICLE 1 – PREDICTING WATER QUALITY IMPACT AFTER DMAS IMPLEMENTATION IN A FULL-SCALE DWDS

The first article presents findings from the investigation of the impacts of DMAs implementation on water quality as well as an approach to predict THMs concentrations in DMAs area using parameters measured at an inlet site. This paper was submitted to *Journal AWWA* and accepted with revisions. The original version is presented here after. Supplementary information is presented in Appendix 2. Additional information on the formation of THMs at chlorinated treatment plant water is presented in Appendix 3.

PREDICTING WATER QUALITY IMPACT AFTER DMAS IMPLEMENTATION IN A FULL-SCALE DWDS

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ABSTRACT

District metered areas (DMAs) with pressure management can reduce leakage and break frequencies as well as extend the pipe's service life in drinking water distribution systems. Valves must be closed to isolate the sectors creating temporary hydraulic disturbances and increasing the number of dead-ends. A full-scale study was conducted during the temporary implementation of five pilot DMAs. Using an enhanced sampling program, water quality was measured at different locations inside and outside DMAs boundaries before and after implementations. Water quality (chlorine, turbidity and metals) is influenced by the closing of valves at locations such as created dead-ends, sites outside DMAs boundaries, and extremities. A site-specific chlorine decay regression model was proposed to predict THM4 concentrations using parameters measured at the DMA inlet site. Results show that utilities can use a combination of hydraulic modeling and targeted monitoring to predict water quality changes after DMA implementation.

KEYWORDS

District metered area; water quality; iron; trihalomethanes; drinking water distribution system; dead-ends

4.1 Introduction

Large amounts of drinking water, ranging from 15 to over 50%, produced worldwide in drinking water treatment plants are wasted due to leakage in drinking water distribution systems (DWDS) (American Water Works Association (AWWA), 2003; Jakubic, 2007; Magini, *et al.*, 2007; Smeets, *et al.*, 2009). Water losses cause significant revenue loss for water utilities (American Water Works Association (AWWA), 2009). The most common causes of leakage are pipe age, materials, human error (workmanship defects, poor installation, mishandling of materials prior to installation), environmental conditions (corrosion, weather, vibration and traffic loading) and operational conditions (incorrect backfill, pressure transients, operating pressure, lack of proper scheduled maintenance) (Thornton, *et al.*, 2008). In North America, aging infrastructure is approaching the end of its useful life (American Society of Civil Engineers (ASCE), 2009) and has been the leading cause of water infrastructure failures compromising system efficiency (Ontario Municipal Benchmarking Initiative (OMBI), 2008; American Water Works Association (AWWA), 2009).

In response, several strategies have been developed to minimize water losses. Unlike system renewal, which is expensive and only possible at long-term, pressure management can be applied relatively quickly and is a cost-effective way to prevent new leaks in existing aging pipe systems and to extend their service life (Fanner, *et al.*, 2007). Moreover, pressure management reduces flow rates from leakage, the frequency of new breaks, and has been successfully used in combination with monitored district metered areas (DMAs) to reduce and identify leaks in DWDS (American Water Works Association (AWWA), 2009). However, this practice involves closing valves creating physical dead-ends and hydraulic changes that may result in lower velocities, changes in flow direction and increased water residence time (WRT), and therefore cause water quality degradation (Brandt, *et al.*, 2004; Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009). The changes associated with the increase of WRT can be chemical, biological, and physical, such as loss of disinfectant residual, disinfection by-products (DBPs) formation, microbial regrowth, sediment deposition, corrosion, discoloration, and taste and odor. Furthermore, dead-ends are typically susceptible to water quality monitoring failures.

Several water utilities have implemented DMAs and reported associated benefits (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009; Kunkel & Sturm, 2011). Despite sectored areas being likely to have high WRT and dead-ends, systems who applied this practice have not reported any significant changes in water quality (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; Kunkel & Sturm, 2011). On the contrary, historical data have shown that DMAs can have an impact on discoloration events and higher customer complaints (Armand, *et al.*, 2015).

The evidence of the impact of DMA implementation on water quality is scarce, with conclusions often supported by limited data. The UK Water Industry Research (UKWIR) verified similar chlorine concentrations (only one measurement) at various locations in six DMAs, including created dead-ends (due to closed valves) and existing dead-ends. Their main findings indicate that DMAs do not seem to have long-term effects on water quality. Short-term effects depend on the susceptibility of the network to cause quality problems, and on average the water quality in the area stays unchanged or slightly improved. However, they did not verify water quality at these sites before the implementations. A Water Research Foundation (WRF) project evaluated water quality in several water utilities in North America, but a comparison between before and after DMAs implementation could only be performed by the Philadelphia Water Department (Fanner, *et al.*,

2007; Kunkel & Sturm, 2011). Chlorine residuals were measured at 10 hydrants inside and 10 hydrants outside the DMA boundary, as well as some water quality parameters at points inside the DMA. Higher chlorine concentrations were measured at most hydrants (17/20) after closing valves and turbidity remained above the accepted range at the furthest point after closing valves. However, the comparison rested on only one measurement before and one six months after the closure of valves from different seasons (winter/summer).

To address these issues, a full-scale field study was conducted during the temporary implementation of five DMAs in a large DWDS. We investigated the impacts of DMAs implementation on water quality from different locations inside and outside boundaries. Sampling points were selected in and out of the DMAs based on their characteristics (new and existing dead-end, inlet, etc.). However, as detailed field investigations may not be practical for all utilities, an approach based on the combination of hydraulic modeling and site-specific chlorine/THM4 correlations was developed to predict THM4 levels in the DMA area using measurements only from an inlet site. The objectives of this study were to: i) verify the differences in water quality before and after DMAs implementation at different types of sites while accounting for the changes in incoming water quality and distribution conditions, ii) evaluate the distribution of chlorine residuals and THM4 formation before and after the setup of the temporary DMAs, iii) develop a relationship between chlorine consumption and THM4 formation as a function of WRT in the DMAs, and iv) propose a simple approach to assess potential risks of water quality deterioration after DMA implementation.

4.2 Materials and methods

4.2.1 Characteristics of the DMAs and sampling strategy

In 2012 and 2013, five pilot DMAs were implemented across a full DWDS serving nearly 1.5 million residents across around 4,000 km of pipes in Montreal (Canada). The distributed drinking water is produced by two treatment plants supplied by surface water and treatment process

included flocculation, filtration, ozonation and post-disinfection with chlorine. Figure 4.1 presents the schematic diagram of the DWDS of this study and the characteristics of the DMAs.

Characteristics		DMA1	DMA2	DMA3	DMA4	DMA5
Total length (km)		64.3	88.2	99	77.5	52.8
# inlets after implementation		3	4	5	6	4
# closed valves		3	31	19	18	14
Frequency of sampling	Before	5	3	3	3	3
	After	5	6	13	3	3
Number of sites sampled		10	12	13	10	10

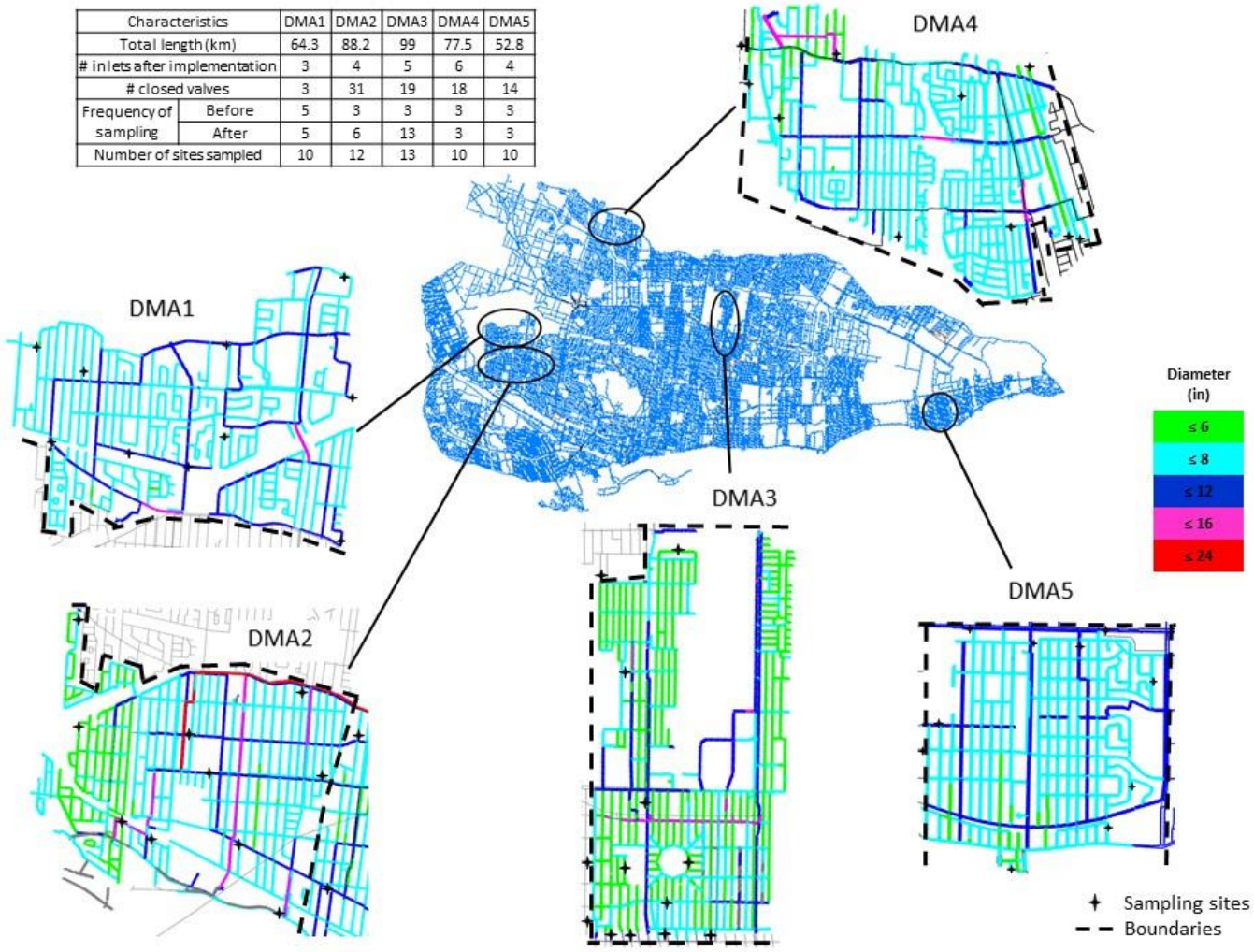

Figure 4.1: DMAs boundaries with pipe diameters and sampling sites locations

The selection of sampling sites was performed to observe changes on water quality by comparing results of hydraulic simulation in order to cover various situations resulting from changes caused by DMAs implementation, based on the characteristics of the DMA, characteristics of the site, and changes on flow direction. Depending on the characteristics and size of DMAs, 10 to 13 sampling points were selected. Each point was sampled weekly, at least three times before and three times after the implementation of DMAs, on the same day of the week and the same time of the day (morning peak) to minimize variations due to consumer demand for water.

Sampling locations were assigned to one of seven location groups: inlets (to establish the baseline for water quality), existing and created dead-ends after the closing of the valves, outside DMA (near the boundaries), extremities (sites far away from the inlet), sites with changes in flow direction or water age variation, and compliance monitoring sampling points used by the water utility. Sampled pipes in these sectors are made of cast iron or ductile iron (at few locations on DMAs 1 and 5) with internal pipe diameters varying from 4 to 14 inches. Because of stagnation and user complaints due to aesthetic problems, flushing was performed at DMA 3 and a valve was partly open at DMA 4 in a created dead-end.

Water quality samples were taken at public buildings (only compliance monitoring sites) and outdoor household taps (both previously disinfected), after refreshing the pipe until the water temperature was constant to confirm that the water from the main pipe had reached the tap. All samples were taken following standard procedures, using sterile bottles, transported to the laboratory at 4°C and processed on the same day.

4.2.2 Water quality analysis

General water quality parameters, such as temperature, pH, chlorine residuals, turbidity and conductivity were measured at the time of sampling with portable instruments according to standardized methods. Free and total chlorine residual measurements were conducted with the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method using a DR/2010 spectrophotometer (HACH) according to Standard Methods 4500-Cl (American Public Health Association (APHA), *et al.*, 2005). DOC samples were filtered through pre-rinsed 0.45 µm hydrophilic polyethersulfone membranes (Supor®-450, PALL, USA), then analysis measurements were conducted on laboratory total organic carbon analyzer (Sievers Instruments, Inc., GE, USA) according to Standard Methods 5310-C (American Public Health Association (APHA), *et al.*, 2005). DBPs formation was verified

by four trihalomethanes (THM4: chloroform, bromoform, bromodichloromethane, dibromochloromethane) and six haloacetic acids (HAA6: monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, dibromoacetic acid and bromochloroacetic acid). THMs were extracted by liquid/liquid extraction with hexane as described in Standard Methods 6232-B (American Public Health Association (APHA), *et al.*, 2005). HAA compounds were extracted by liquid/liquid extraction with methyl tert-butyl ether (MtBE) followed by derivation with acidic methanol in accordance with USEPA Method 552.2 (United States Environmental Protection Agency (USEPA), 1995). All DPBs were analyzed on a gas chromatograph (CP-3800, Varian) equipped with an electron capture detector (GC/ECD) (Column characteristics: capillary column RTX-5MS with 30 m × 0.25 mm ID and 0.25 μm film thickness). Iron and manganese (dissolved and total for both parameters) were analyzed by ICP (Thermo Fisher iCAP 6000) (American Public Health Association (APHA), *et al.*, 2005). Analyses were performed in duplicate or triplicate and averaged for each sample.

4.2.3 Hydraulic network model

Hydraulic simulations were conducted using calibrated models provided by the water utility to assess the impact of DMAs on parameters that can influence the water quality such as the WRT, water velocities, and changes in flow direction to guide the choice of sampling sites. Simulations were performed on WaterGEMS V8i software with scenarios before and after DMAs implementation using average daily consumption considering hourly variations patterns. In order to assure stability and repeatability, the simulation was carried out over 15 days using extended period simulation and the values obtained from day 10 at the minimum and maximum water demand conditions were considered.

4.2.4 Approach to predict THM4 concentrations and validation

The objective of the modeling approach was to predict THM4 concentrations at various sites in the DMA area (site indexed *j*) using only data from an inlet site. To this end, all data collected from the first four DMAs (DMAs 1-4) were merged together to construct the predictive model and the data from DMA 5 was used for validation. Samples were paired such that each downstream site with a value of WRT (site *j*) was assigned to an upstream site with low value of WRT (sites *i*) for each sampling day. pH, temperature (T), free chlorine (Cl_2), DOC, and THM4 at sites *i* as well as

the WRT difference between sites *i* and *j* (ΔWRT_{ij}) were tested as independent variables to predict THM4 at sites *j*:

$$THM4_j = a_0 + a_1 pH_i + a_2 T_i + a_3 Cl_{2_i} + a_4 DOC_i + a_5 THM4_i + \Delta WRT_{ij} \quad (1)$$

The predictive model was constructed using multiple stepwise linear regression in STATISTICA 64 software v.13 using data from DMAs 1-4 and was validated using an inlet site from DMA 5.

4.2.5 Data analysis

Free chlorine consumption, THM4 formation, and WRT variation were calculated by the difference between the concentrations at the sites and their respective inlet. Correlations between these variables were obtained by linear regression. The strength of the associations between water quality parameters was calculated using Pearson's correlation method for all data combined (DMAs 1-5). Analyses were done using STATISTICA 64 software v.13.

4.3 Results

4.3.1 Changes in water quality after DMAs implementation along different sites

Water quality before and after DMAs implementation across different sampling points are presented in Figures 4.2 to 4.5 and in Appendix 2 (Figures A-2.1 to A-2.3). The sampling sites were grouped to account for the variation of each type of site (Table 4.1). Considerable variability in water quality occurred in the distribution system during the period along the five DMAs at the monitored sampling points: pH values ranged from 7.7 (DMAs 1, 2, and 3) to 8.3 (DMAs 2, 4, and 5), temperature from 8.3°C (DMA 2) to 23.9ºC (DMA 3), turbidity from 0.11 NTU (DMAs 1, 3, and 5) to 1.06 NTU (DMA 4), DOC from 1.8 mg/L (DMA 1) to 3.3 mg/L (DMA 3), free chlorine residuals from 0.01 mgCl_2/L (DMAs 1 and 5) to 1.12 mgCl_2/L (DMAs 2 and 4), THM4 from 11.7 µg/L (DMA 2) to 89.9 µg/L (DMA3), HAA6 from 0 (DMA 4) to 62.7 µg/L (DMA 3), total iron from 1.9 µg/L (DMA 1) to 413.7 µg/L (DMA 2), and total manganese from 0.1 µg/L (DMA 5) to 13.7 µg/L (DMA 3).

Concentrations at inlets serve as the baseline values for the water quality measured at each DMA. During the monitoring, notable changes were noted at some inlets for temperature (DMAs 1-3 and

5), DOC (DMAs 2 and 3), free chlorine residuals (DMAs 1, 2, and 4) and THM4 (DMAs 3-5). These differences reflect changes occurring in temperatures (season variations) and/or in treatment at plants (e.g., chlorine dosage). Additionally, the impact on valve closing was only evident at some types of sampling points. Generally, parameters such as pH, temperature, and DOC at sites inside/outside DMAs followed trends at inlets (Figures A-2.1 and 4.2, respectively). Changes were detected for turbidity, free chlorine residuals, and metal concentrations. Turbidity values (Figure 4.2) were higher at created dead-ends (DMAs 2, 3, and 5), outside sites (DMAs 2 and 3), and a compliance monitoring site (DMA 2) after DMAs implementation (maximum mean increase of 0.35 NTU at created dead-ends in DMA 5), while existing dead-ends presented more stable concentrations. Generally, lower values were observed at DMAs 1 and 4, as well as at the extremities of DMA 2, probably influenced by the lower velocities after valve closing.

Table 4.1: Number of sampling sites in each DMA by type before and after implementations

Types of sampling sites	**DMA 1**	**DMA 2**	**DMA 3**	**DMA 4**	**DMA 5**
Inlet Before	3	3	2	4	2
Inlet After					
Existing dead-end Before	4	-	1	2	2
Existing dead-end After					
Created dead-end Before	-	4	3	1	1
Created dead-end After					
Outside DMA Before	-	2	2	1	1
Outside DMA After					
Extremity Before	2	1	1	1	-
Extremity After					
Changes in flow or water residence time Before	-	-	3	1	3
Changes in flow or water residence time After					
Compliance monitoring sites Before	1	2	1	-	1
Compliance monitoring sites After					

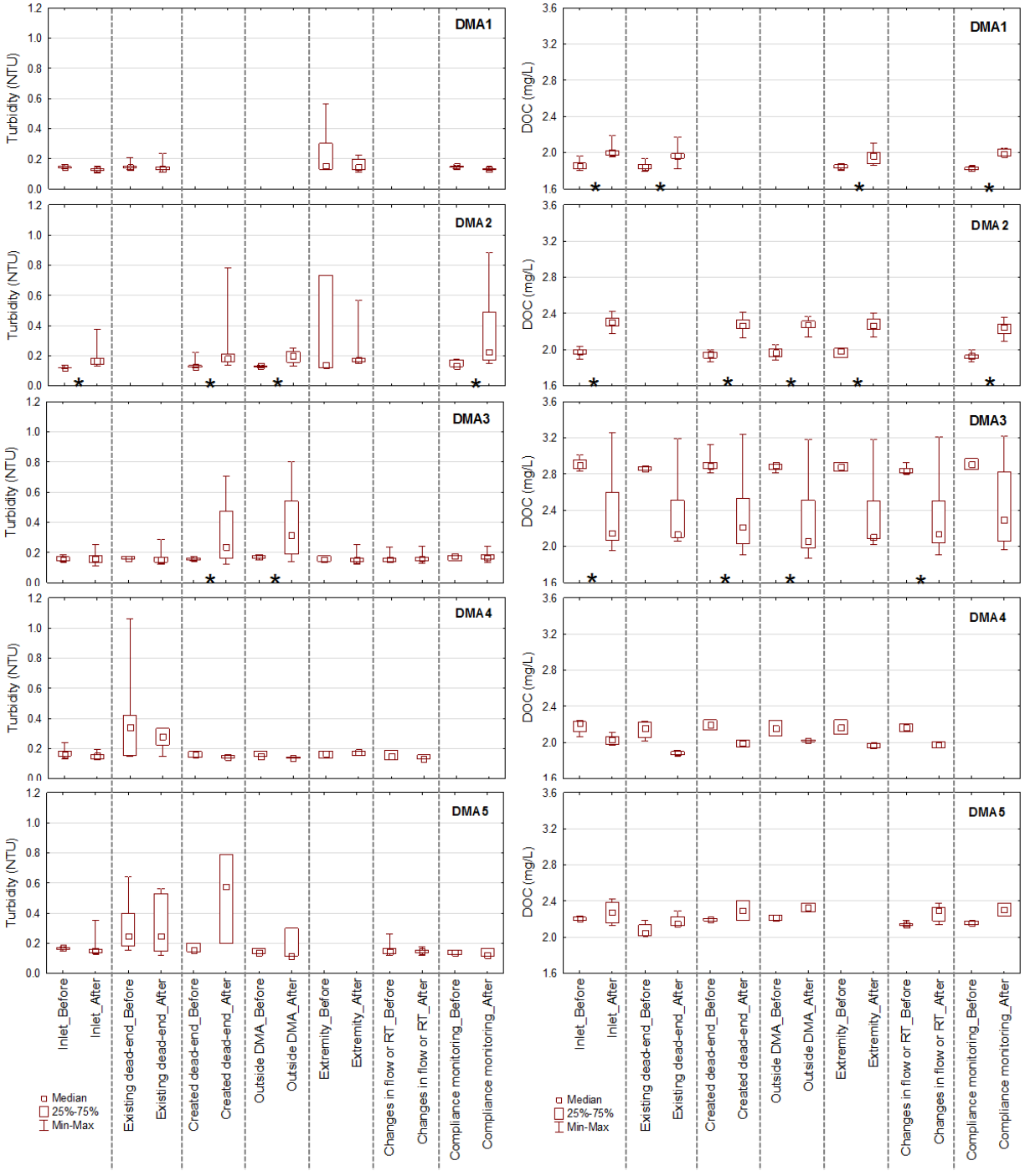

Figure 4.2: Box-and-whisker plots of turbidity and dissolved organic carbon (DOC) across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values. Before and after groups for each type of site are statistically different at $\rho<0.05$ by Wald-Wolfowitz Test (*)

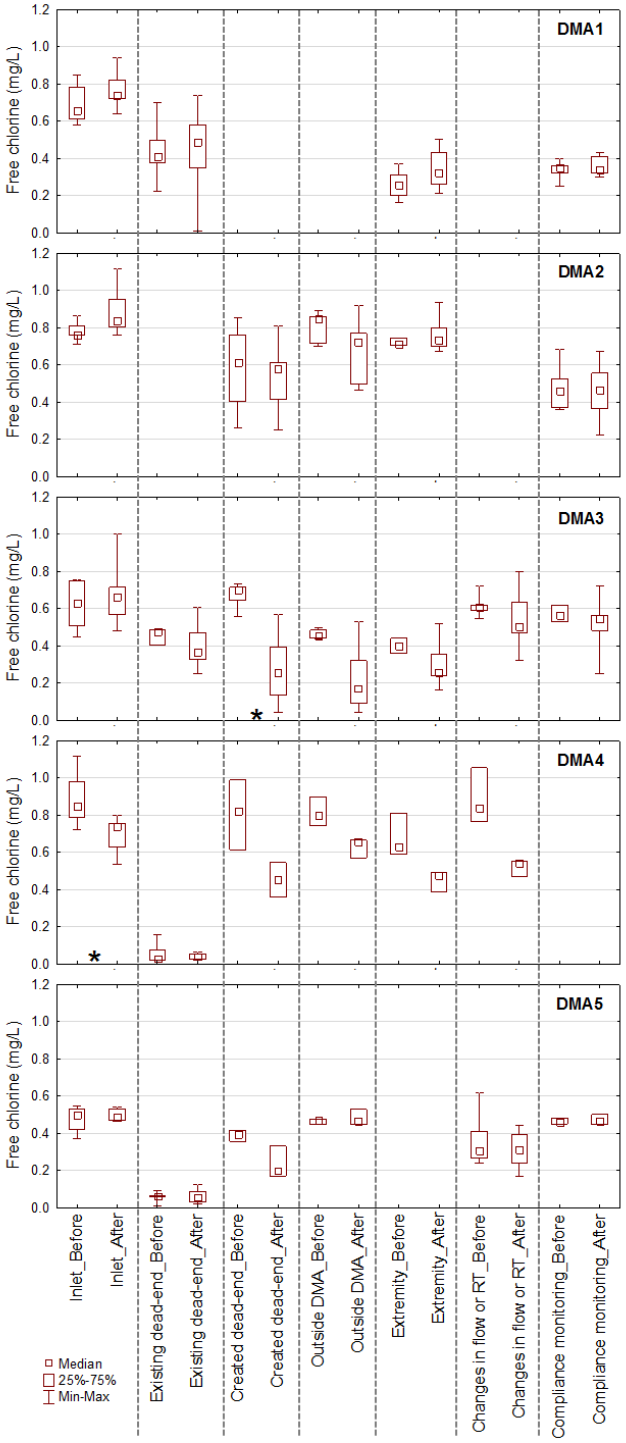

Figure 4.3: Box-and-whisker plots of free chlorine residuals across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values. Before and after groups for each type of site are statistically different at $\rho<0.05$ by Wald-Wolfowitz Test (*)

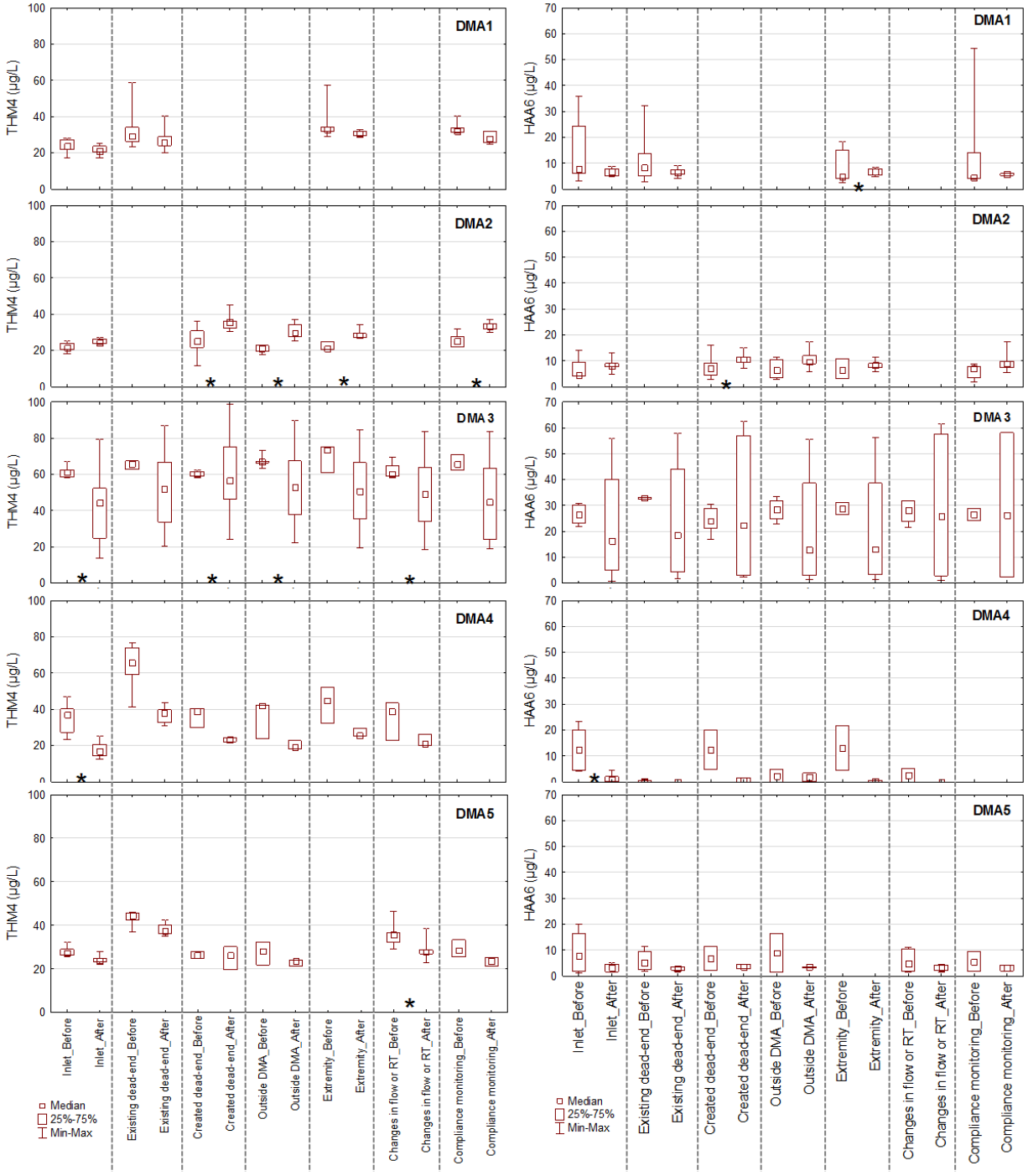

Figure 4.4: Box-and-whisker plots of trihalomethanes (THM4) and haloacetic acids (HAA6) across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values. Before and after groups for each type of site are statistically different at $\rho<0.05$ by Wald-Wolfowitz Test (*)

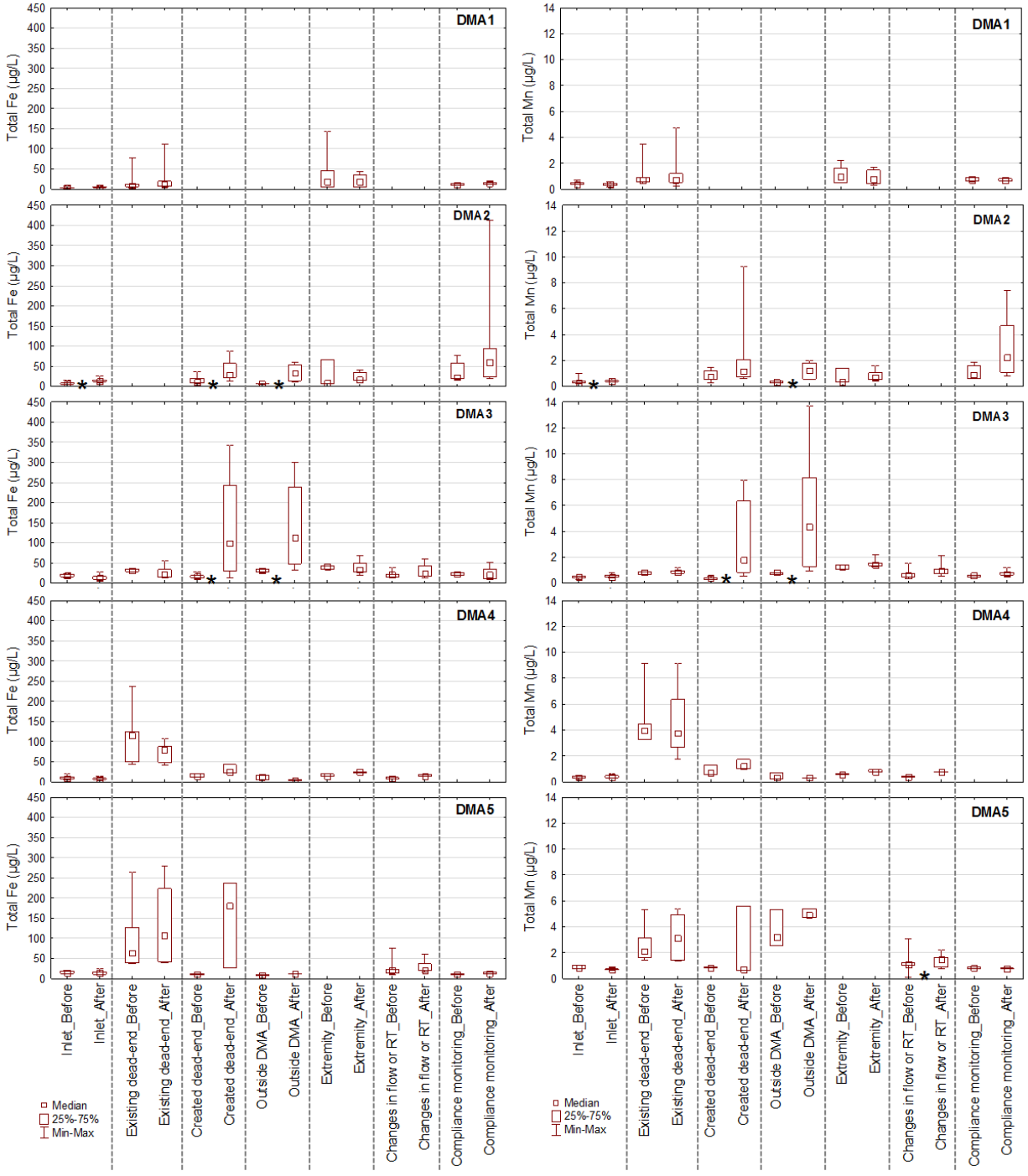

Figure 4.5: Box-and-whisker plots of total iron (Fe) and manganese (Mn) across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values. Before and after groups for each type of site are statistically different at $\rho<0.05$ by Wald-Wolfowitz Test (*)

Free chlorine residuals (Figure 4.3) were notably lower at created dead-ends (DMAs 3, 4 and 5), outside sites (DMAs 3 and 4), extremities (DMA 4), and sites with changes in flow direction or WRT (DMA 4). Lower concentrations were also observed at inlets of DMA 4. At DMA 3, created dead-ends and outside sites presented, at some time after implementations, free chlorine concentrations very similar to existing dead-ends with stagnation (e.g., existing dead-ends at DMAs 4 and 5 with mean concentrations around 0.06 $mgCl_2/L$). At DMA 2, even if inlets presented a small increase (mean increase of 0.1 $mgCl_2/L$) in chlorine concentrations and temperatures were lower (mean decrease of 5.4ºC) after the implementation, lower after values (mean reductions of 0.06 $mgCl_2/L$ and 0.13 $mgCl_2/L$, respectively) were observed at created dead-ends and outside sites.

Changes in mean WRT (Figure A-2.2, Appendix 2) were modest as the sampling occurred during the morning peak consumption and the calibrated model presented a water demand in most dead-ends. Significant changes were noted at the points near the new boundaries and the created dead-ends.

THM4 at inlets varies between 20 μg/L and 40 μg/L, except for DMA 3 (13.7-79.5 μg/L) as sampling coincides with seasonally higher DOC levels (up to 3.3 mg/L). There were no trends by type of sampling point or impact after the implementations. Concentrations in each DMA seem to follow inlet variations. HAA6 levels were normally under 20 μg/L in all DMAs, except for DMA 3 (Figure 4.4) when DOC concentrations were higher (Figure 4.2). Their variations also followed THM4 deviations.

Iron and manganese values (Figure 4.5) evolved similarly and followed the trends observed for turbidity. Existing dead-ends presented high values during all the monitoring at DMAs 1, 4, and 5, while some created dead-ends and outside sites presented elevated values after implementations (DMAs 2, 3, and 5). At DMA 2, compliance monitored sites presented elevated values during the monitoring. These sites were sampled inside buildings and the premise plumbing seems to influence iron and manganese values even if the pipe was refreshed and temperature was constant.

Existing compliance monitoring sites could not detect the water quality changes seen in other impacted types of sites, and therefore would not be appropriate to monitor water quality changes caused by DMA implementations. Also, as these samples are collected in taps inside public buildings, their quality could be influenced by premise plumbing conditions.

4.3.2 Distribution of free chlorine residuals and THM4 before and after DMAs implementation

Figure 4.6 shows the cumulative distribution of free chlorine residuals and THM4 before and after implementation of DMAs at the various types of sampling sites. Trends vary depending on the DMA considered. In DMAs 1 and 4, the distribution of chlorine residuals followed the general trends at inlets but was higher or lower. In DMAs 3 and 5, with comparable before/after chlorine residuals at inlets, the distributions after implementation reveal clearly lower chlorine concentrations. The distribution of chlorine residuals also decreased in DMA 2 after the implementation even if slightly higher after values at inlets were observed. A trend towards the loss of chlorine residuals (<0.2 $mgCl_2/L$) after implementations was most important in DMAs 3 and 5 (decrease of 20% and 13%, respectively). Distributions of THM4 show higher values after implementation in DMA 2 (with comparable before/after THM4 at inlets) and for concentrations more elevated than 70 µg/L in DMA 3. DMAs 1 and 5 showed before THM4 distribution higher than the after ones with similar before/after THM4 concentrations at inlets. In DMA3, concentrations were always below 80 µg/L before but 14% of the measured THM4 were above this limit after implementation.

4.3.3 Relationship between free chlorine consumption, THM4 formation and water residence time

Table 4.2 presents the linear models predicting free chlorine consumption and THM4 formation with WRT variation in each DMA and summarizes the range of some water quality parameters measured during the period considered. Free chlorine consumption per 10 h of WRT ranged between 0.12 $mgCl_2/L$ (DMA 5) to 0.27 $mgCl_2/L$ (DMA 3). THM4 formation per 10 h of WRT variation ranged from 3.7 µg/LTHM4 (DMA 5) to 7.2 µg/LTHM4 (DMA 3). The lowest values for DMA 5 can be explained by lower chlorine concentrations in this sector compared to the others. Higher chlorine and DOC concentrations influenced the higher relationships for DMA 3. Differences between DMAs 1 and 3 with DMA 4 (4.7, 5.5 and 7.0 µg/LTHM4, respectively) can be explained by higher WRTs and/or temperature in this sector. Regression coefficients between chlorine consumption and THM4 formation ranged between 19.3 $\mu gTHM4/mgCl_2$ (DMA 1) and

31.0 µgTHM4/mgCl_2 (DMA 4). These correlations were influenced by dead-end sites with higher chlorine consumption, and subsequently THM4 formation.

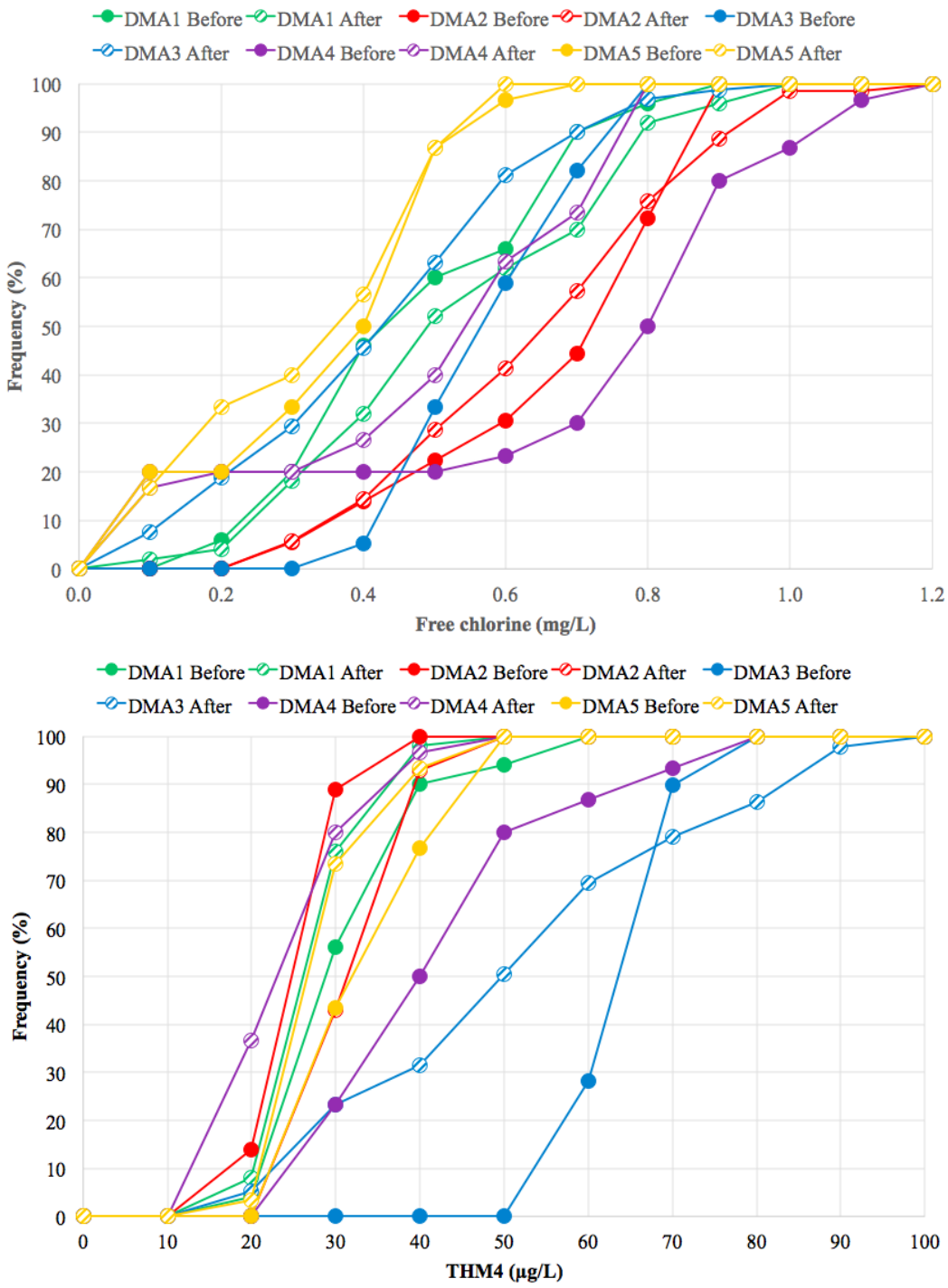

Figure 4.6: Distribution of chlorine residuals and THM4 concentrations at DMAs sampling locations before and after the implementations

Table 4.2: Correlations between free chlorine consumption, THM4 formation, and water residence time variations in each DMA

	Regression slope coefficient, R^2 and number of samples (n)			Range of concentrations of water quality parameters					
DMA	**Free chlorine consumption / 10 h WRT variation ($mgCl_2/L$)**	**THM4 formation / 10h WRT variation (µgTHM4/L)**	**THM4 formation/ free chlorine consumption ($\mu gTHM4/mgCl_2$)**	**pH variation**	**Temperature variation (°C)**	**Free chlorine variation (mg/L)**	**DOC variation (mg/L)**	**THM4 variation (µg/L)**	**WRT variation (h)**
1	0.24 (R^2=0.70 / n=90)	4.7 (R^2=0.51 / n=90)	19.3 (R^2=0.68 / n=90)	7.7-8.1	13.8-23.1	0.01-0.94	1.8-2.2	17.3-40.5	11.2-37.3
2	0.23 (R^2=0.75 / n=106)	5.5 (R^2=0.61 / n=106)	23.3 (R^2=0.67 / n=106)	7.7-8.3	8.3-19.2	0.23-1.12	1.9-2.4	11.7-44.9	9.4-32.2
3	0.27 (R^2=0.78 / n=148)	7.2 (R^2=0.44 / n=89)	28.7 (R^2=0.62 / n=119)	7.7-8.1[a,b] 7.7-8.2[c]	13.6-23.9[a,b,c]	0.04-1.00[a,b,c]	1.9-3.3[a,b,c]	13.7-89.9[a,b] 13.7-98.7[c]	10.7-33.2[a,b] 10.7-176[c]
4	0.22 (R^2=0.88 / n=59)	7.0 (R^2=0.86 / n=58)	31.0 (R^2=0.90 / n=58)	7.9-8.3	15.8-22.9	0.02-1.12	1.9-2.3	12.5-76.5	8.6-52.4
5	0.12 (R^2=0.78 / n=58)	3.7 (R^2=0.75 / n=49)	30.5 (R^2=0.82 / n=48)	8.0-8.3	11.6-20.5	0.01-0.55	2.0-2.4	19.6-46.5	15.2-56.8

a – range of concentrations for free chlorine consumption / 10 h WRT variation at DMA 3

b – range of concentrations for THM4 formation / 10 h WRT variation at DMA 3

c – range of concentrations for THM4 formation / free chlorine consumption at DMA 3

4.3.4 Correlations between water quality parameters across DMAs

Table A-2.1, presented in Appendix 2, lists Pearson's correlations between measured water quality parameters for all datasets combined (DMAs 1-5). Free chlorine values were strongly correlated to metals and moderately correlated ($0.3<r<0.5$) to temperature, turbidity, THM4 and WRT. THM4 levels were strongly correlated to DOC, HAA6 and dissolved iron, and moderately correlated with turbidity, WRT and metals. HAA6 concentrations were also strongly correlated to DOC and moderately correlated to pH. Metals were also strongly correlated to turbidity. The correlations between iron and manganese concentrations and turbidity in DMAs 1-5 are shown in Figure 4.7. Concentrations of these metals were clearly correlated with turbidity as also seen in Table A-2.1 (Appendix 2).

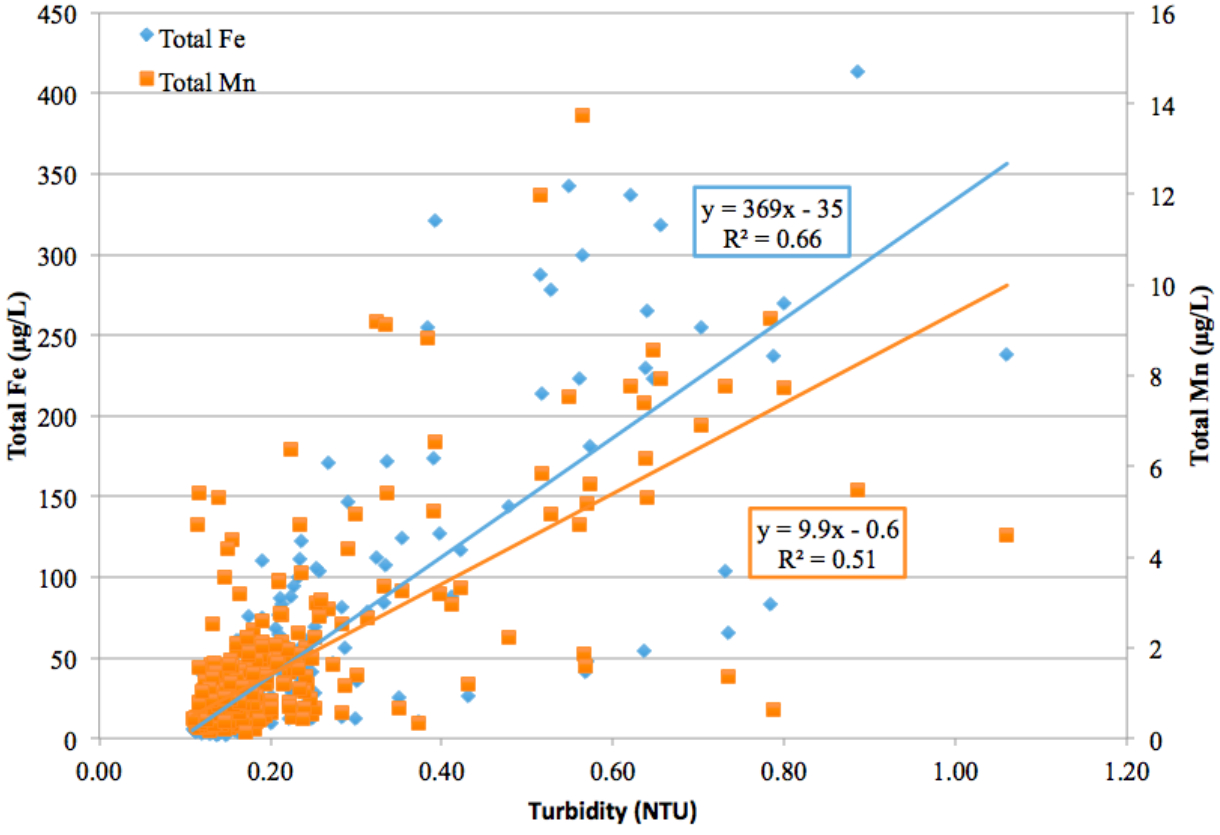

Figure 4.7: Total iron and manganese concentrations versus turbidity values at DMAs1-5

4.3.5 Predictive THM4 approach and validation

Table 4.3 lists the results from the multiple linear regression performed on the 169 observations from DMAs 1-4 to predict THM4 concentrations at a site *j* from the values measured at an inlet site (*i*). The best R^2 (0.91) was found using non-transformed data and the significant variables were free chlorine and THM4 concentrations at upstream sites and the variation of WRT between the sites. Predicted THM4 *vs*. observed THM4 values from the regression model is presented in Figure A-2.4 (Appendix 2). Subsequently, the model was validated using data (free chlorine, THM4, and WRT values) from one inlet site at DMA 5 and predicting THM4 values for the six sampling dates at all nine other sites using only the WRT from these sites. Predicted THM4 *vs*. measured THM4 values in DMA 5 show an excellent fit for THM4 values (Figure A-2.4, Appendix 2). This model is valid for free chlorine concentrations ranging between 0.25 mgCl_2/L and 1.12 mgCl_2/L, THM4 values ranging from 11.7 µg/L to 83.5 µg/L at *i* sites; predicted THM4 from 19.3 µg/L to 89.9 µg/L, and ΔWRT_{ij} from 0.10 to 41.4 h.

Table 4.3: Results for the multiple linear regression performed on 169 observations from four different DMAs to predict THM4 concentrations at a site *j* from the values measured at an inlet site (*i*)

Observations	**R^2**	**Adjusted R^2**	**F (3,169)**	**ρ-value**
169	0.911	0.910	579.63	<0.001
Variables	**Coefficient**	**Standard error of the coefficient**	**t (169)**	**ρ-value**
Cl_2_*i*	6.658	2.335	2.852	<0.005
THM4_*i*	1.062	0.026	41.128	<0.001
ΔWRT	0.578	0.045	12.852	<0.001
Intercept	-5.814	1.980	-2.937	<0.005

Cl_2_*i*: free chlorine concentration at inlet (mg/L); THM4_*i*: concentration of THM4 at inlet (µg/L); ΔWRT: difference between the water residence times at inlet and a site *j* in the area of the DMA

4.4 Discussion

Water quality was monitored at various types of sampling points before and after the temporary setup of five DMAs in a full-scale DWDS. The five studied DMAs included different characteristics, lengths of pipes, and types of sampling sites inside/outside the boundaries. Moreover, the study was performed over two years spanning over three seasons (spring, summer, and fall). Previous studies investigating impacts on water quality associated with implementing DMAs established their conclusions using scarce evidence from limited monitoring campaigns (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; Kunkel & Sturm, 2011). Our study deployed an enhanced monitoring plan at full-scale field with repeated sampling at sites selected to show changes under constant or variable hydraulic conditions. This investigation was carried out taking into account the hydraulic characteristics of different sampling points. Additionally, by measuring water quality at the inlets, it was possible to distinguish the changes in water quality mainly related to temperature and those following seasonal patterns or fluctuations in treatment at plants (e.g., chlorine dosage, DOC concentrations).

Changes in hydraulic conditions (WRT, water velocities and flow direction) are expected following the closing of valves to form DMA boundaries, which can produce short-term and permanent impacts on water quality. The extent of these changes depend on the extent of the shifts and must can only assessed with proper pairing of samples according to their hydraulic conditions. Our monitoring results demonstrate that closing valves to create DMA boundaries can lead to some deterioration at sites with increased and elevated WRT (created dead-ends, outside sites near the boundaries, and extremities). The parameters most influenced by DMAs implementation were chlorine residuals, turbidity, iron and manganese. Results also evidenced the trends in water quality at inlet points that were most influenced by the season and/or treatment. Other parameters such as temperature, pH, DOC, THM4 and HAA6 followed trends at inlet points and were clearly more related to changes of seasonal WQ and/or treatment.

Disinfectant decay normally occurs with time due to interactions with substances in bulk water, biofilm, and pipe material. Maintaining a chlorine residual from the treatment plant to the extremities within a DWDS is challenging, especially if it must be achieved without excessive dosage and multiple rechlorination (Prévost, *et al.*, 2014). Higher dosages at plants and/or rechlorination could cause taste and odor issues as well as additional DBPs formation (e.g. THMs

and HAAs), mainly in warmer months as the reactions proceed faster at higher temperatures (Rodriguez, *et al.*, 2007). Dead-ends and extremities at water systems are known to be the locations the most susceptible locations to present very low or even undetectable disinfectant residuals due to stagnation and higher WRT. Results from our study demonstrated that the implementation of DMAs could increase the number of locations with low chlorine residuals being similar to those found at existing dead-ends in the system.

DBPs are a concern in drinking water because of their potential health effects including increased risk of bladder cancer and reproductive implications (Sadiq & Rodriguez, 2004; Richardson, *et al.*, 2007; Villanueva *et al.*, 2015). Factors influencing the formation and levels of DBPs include water quality parameters such as pH, temperature, concentration and reactivity of organic matter, treatment, chlorination conditions, and seasonal variations (Liang & Singer, 2003; Sadiq & Rodriguez, 2004; Brown, *et al.*, 2011). THMs and HAAs are the two groups of chlorinated DBPs most often regulated under various international legislations (Richardson, *et al.*, 2007). Their levels in DWDS vary according to water source, water treatment, rechlorination, season, and WRT (Rodriguez, *et al.*, 2007; Simard, *et al.*, 2011). The US standard for THM4 and HAA5 is assessed on the basis of and annual average of 80 μg/L and 60 μg/L, respectively (United States Environmental Protection Agency (USEPA), 2006). Concentrations of THMs and HAAs above these limits imposed by the regulation were observed only at DMA 3. These short-term spikes were associated with higher chlorine dosages as well as elevated DOC concentrations in the water entering the inlets. The results obtained for DBPs in the five DMAs of this study follow DBP trends and DOC concentrations at inlets and only minor differences were observed after DMAs setup.

The extensive monitoring demonstrated that DMA implementation can impact iron and manganese levels in the created dead-ends and the outside DMA sites near the boundaries. Some spikes exceeding the recommended levels for iron were observed at one created dead-end (DMA 3) and at one compliance monitoring site (DMA 2), both after DMAs implementation. Moreover, a yellow color was noted in one created dead-end and outside samples in DMA 3 during two or three weeks after the implementations probably caused by elevated iron concentrations and poor chlorine residuals. Iron and manganese are not considered to present a risk to human health, but they are listed in the non-mandatory water quality standards recommended by US EPA (United States Environmental Protection Agency (USEPA), 2016). A secondary maximum contaminant level for these metals of 300 μg/L for iron and 50 μg/L for manganese are suggested in order to prevent

aesthetic issues. However, recent epidemiological studies suggested significant neurological effect in children associated with manganese exposure in drinking water (Wasserman, *et al.*, 2006; Oulhote, *et al.*, 2014).

Higher concentrations of total iron and manganese and elevated turbidity were measured at existing dead-ends, created dead-ends and extremities as shown in Figures 4.2 and 4.5 and this relationship is illustrated by Figure 4.7. Concentrations of these metals were clearly correlated with turbidity as also seen in Table A-2.1 (Appendix 2) for all DMAs combined. Samples were collected at the morning demand peak for feasibility reasons. However, higher flow velocities caused by the consumption may have released accumulated particles of iron and manganese from the pipe walls causing discoloration. Higher concentrations of iron and manganese in bulk water can result in discoloration events (Slaats, *et al.*, 2003; Vreeburg & Boxall, 2007). A flushing program was completed in the temporary DMAs before implementation. However, it has been shown that the removal of sediments (mostly constituted of iron, organic matter, and manganese) by spot flushing in dead-ends produced short-term impacts in the aesthetic characteristics of the water (Barbeau, *et al.*, 2005). Moreover, studies investigating historical data of DMAs in the UK concluded that DMAs could influence discoloration (Armand, *et al.*, 2015) as well as significantly higher customer complaints during summer when water consumption and water temperatures increase (Prasad & Danso-Amoako, 2014).

Utilities implementing DMAs need to assess the potential impact of sector isolation on the future distribution of regulated WQ parameters such as chlorine residuals and DBPs. Most of the predictive models reported in the literature were developed using empirical relationships derived from linear and non-linear regression analyses relating water quality parameters to DBP concentrations (Sadiq & Rodriguez, 2004; Brown, *et al.*, 2011). The explanatory variables typically include a representative of NOM, pH, temperature, bromide concentrations, chlorine dose or residual, and reaction time. A large number of the predictive models was established from laboratory studies at conditions not normally found in water systems (Brown, *et al.*, 2011). Models based on field data are more realistic even if generally specific for the case that served for the model development and the range of independent variables (Sadiq & Rodriguez, 2004). The simple approach proposed herein relating chlorine consumption to the formation of THM4 in the DMA areas explained 91% of the variability of THM4 concentrations (Table 3). Such a site-specific model accounts for the spatial and pipe effects in the distribution that influence chlorine decay and

THM4 formation. Other WQ variables such as pH, temperature, and DOC did not improve the prediction, as these parameters are already accounted for in chlorine demand. This approach is a simplified tool for the water utility to predict THM4 in other DMAs using only measurements from an inlet site in this distribution system thus minimizing WQ sampling efforts.

4.5 Conclusions

Full-scale field monitoring was conducted during the temporary implementation of five pilot DMAs to document water quality inside and outside DMAs boundaries. This investigation was carried out using an enhanced water monitoring sampling plan that takes into account the hydraulic characteristics of the sampling points with an effort to compare them in terms of hydraulic conditions before and after the isolation of the sector.

Major observations include:

- Water quality entering the DMAs varies significantly and should be accounted for when assessing the impact of DMA implementation.
- Created dead-ends and sites near boundaries outside of the DMAs were the locations most affected by DMAs implementation. The most influenced water quality parameters were disinfectant residuals, turbidity, and metal concentrations.
- Most created dead-ends and outside sites showed water quality similar or worst than those observed in monitored existing dead-ends.
- DMAs implementation augments the number of sites where discoloration events were observed.
- Changes in THM4 concentrations observed after the implementations were attributed to variations in treated water quality entering the sectors.
- A simple approach combining hydraulic modeling and site-specific chlorine demand/THM formation can be used to predict the future distribution of THM4 in a DMA area.
- A program to monitor water quality at dead-ends is desirable. No specific water quality monitoring effort is needed after the implementation of a DMA other than that related to the management in dead-ends.

Our results show that DMAs should be designed to minimize, as much as possible, the number of stagnant sites. The risk of potential water quality issues when implementing DMAs can be

quantified by combining hydraulic modeling and documenting the quality at existing high risk sites by targeted monitoring. Finally, no significant water quality impact following DMAs implementation was observed. Further research is needed to understand the impact of pressure management and the influence of demand conditions in water quality.

4.6 Acknowledgements

This study was supported by the partners of the NSERC Industrial Chair on Drinking Water. The authors thank the Chair staff, especially Mireille Blais, Yves Fontaine, Jacinthe Mailly, and Julie Philibert. The authors would also to thank the utility technical personnel of the City of Montreal for providing hydraulic model and support, and Bentley systems for providing academic access to the utility model.

CHAPTER 5 ARTICLE 2 – ASSESSING THE IMPACT OF DMA IMPLEMENTANTION ON BACTERIAL WATER QUALITY IN A FULL-SCALE DS

This chapter reports the results from the investigation on the impact of district metered areas (DMAs) implementation on bacterial abundance and community structures across different types of sampling locations taking into account their hydraulic characteristics (flow velocity and direction, water residence time). Furthermore, these observations were paired with water quality parameters. The information presented in this paper completes the analysis of the data on other hydraulic and water quality parameters presented in Article 1 (Chapter 4). This chapter was submitted as a journal article to Plos One. Supplementary information is presented in Appendix 4.

ASSESSING THE IMPACT OF DMA IMPLEMENTATION ON BACTERIAL WATER QUALITY IN A FULL-SCALE DS

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ABSTRACT

Pressure management applied to district metered areas (DMAs) reduces leakage losses and new breaks frequencies. The impact of DMA implementation on bacterial quality was measured by monitoring bacterial abundance across the distribution system and community structures at the DMA inlet points and in dead-ends. Observations were paired with water quality sampling (pH, DOC, turbidity, particles, temperature, chlorine residuals, Mn, Fe) before and after the implementation of five full-scale pilot DMAs. Simulations showed minor changes of overall water

residence time, but a significant increase (10-40%) of locations with high residence time (>50h) in 4/5 DMAs. DMA implementation increased HPCs and total bacterial cell counts in newly created dead-ends. Bacterial diversity was related to factors affecting viability and growth of microorganisms (Cl_2 residual, DOC, Fe, etc.) in existing and newly created dead-ends. This suggests that bacterial abundance and diversity reflects local water quality and that utilities can anticipate bacterial water quality changes based on water quality information in existing distribution system critical points and hydraulic simulations.

5.1 Introduction

The implementation of district metered areas (DMAs) in combination with pressure management has been successfully used to reduce losses, new breaks frequencies and to identify leaks in drinking water distribution systems (DS), to extend the life of pipes and to reduce overall costs of system renewal (Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009; Kunkel & Sturm, 2011). The impact of DMA implementation on water quality has been studied by some utilities through field investigations (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009; Kunkel & Sturm, 2011). However, the studies available are limited in scope and duration being either conducted only after the implementation, or only once before and after the implementation.

The implementation of DMAs involves the closing of valves creating physical dead-ends and hydraulic changes that may result in increased water residence times, stagnation, and therefore potential water quality degradation (United States Environmental Protection Agency (USEPA), 2002a; Brandt, *et al.*, 2004; Fanner, *et al.*, 2007; American Water Works Association (AWWA), 2009). Microbial water quality may deteriorate with increased water residence time and stagnation when conditions for regrowth are present (Maul, *et al.*, 1985; Prévost, *et al.*, 1998; United States Environmental Protection Agency (USEPA), 2002b; Zhang & DiGiano, 2002; Prévost *et al.*, 2005; Machell & Boxall, 2014). Regrowth is most often managed through the removal of nutrients and the maintenance of disinfectant residuals throughout distribution. Factors affecting bacterial water quality in DS are complex and include water quality (temperature, pH, concentration of organic and inorganic compounds, biodegradable organic carbon, disinfectant residuals, nitrification,

particles, etc.) and pipe materials (Laurent *et al.*, 2005b; Prévost, *et al.*, 2014). However, bacterial regrowth and the proliferation of resistant pathogens can occur in low nutrient networks or in the presence of a residual disinfectant (Prévost, *et al.*, 1998; Zhang & DiGiano, 2002; Berry, *et al.*, 2006; Liu, *et al.*, 2013; Prévost, *et al.*, 2014). Under low flow conditions, organic and inorganic particles existing in the bulk water can settle and accumulate in pipes. Microorganisms present in sediments can grow protected from disinfectant and be suspended when variations in water velocities occur (Gauthier, *et al.*, 1999; Barbeau, *et al.*, 2005) causing red water issues and consumer complaints (Vreeburg & Boxall, 2007). Investigations on historical data in system implementing DMAs reveal the occurrence of discoloration events and the accumulation of metal particles, such as iron and manganese (Prasad & Danso-Amoako, 2014; Armand, *et al.*, 2015).

Finally, the documented persistence and proliferation of opportunistic pathogens (OPPs) in DSs and associated health impacts justify a reevaluation of the DS biostability, a concept introduced in the 1990s, to include the diversity of the bacterial biomass (Water Research Foundation (WRF) *et al.*, 2013; Prévost, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015). The use of more advanced techniques, such as high-throughput sequencing (HTS) or similar procedures have been used in combination with traditional methods to help better understand bacterial diversity in drinking water DS (Pinto, *et al.*, 2012; Liu, *et al.*, 2014; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015). The impact of residence time on planktonic bacterial communities has been studied in a full-scale unchlorinated DS and few changes were noted at higher residence times (≈ 50 hours) (Lautenschlager, *et al.*, 2013). Other studies at full-scale have focused on comparing communities present at various stages in the treatment plant, the treated water and at locations of the DS, but no particular attention was given to the influence of residence time or stagnation (Pinto, *et al.*, 2012; Lautenschlager, *et al.*, 2013; Liu, *et al.*, 2014; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015). In these investigations, the planktonic bacterial composition remained relatively stable across the DS. Our study investigates the short and medium-term impacts of DMAs implementation on community profiles at full scale, with a specific focus on the impact of water quality on bacterial communities in dead-end sites before and after the implementation of DMAs.

The objectives of this study were to: (1) document the overall distribution of hydraulic parameters before and after DMA implementation to identify the proportion of sites with elevated water residence times; (2) monitor the evolution of HPCs and total bacterial counts before and after

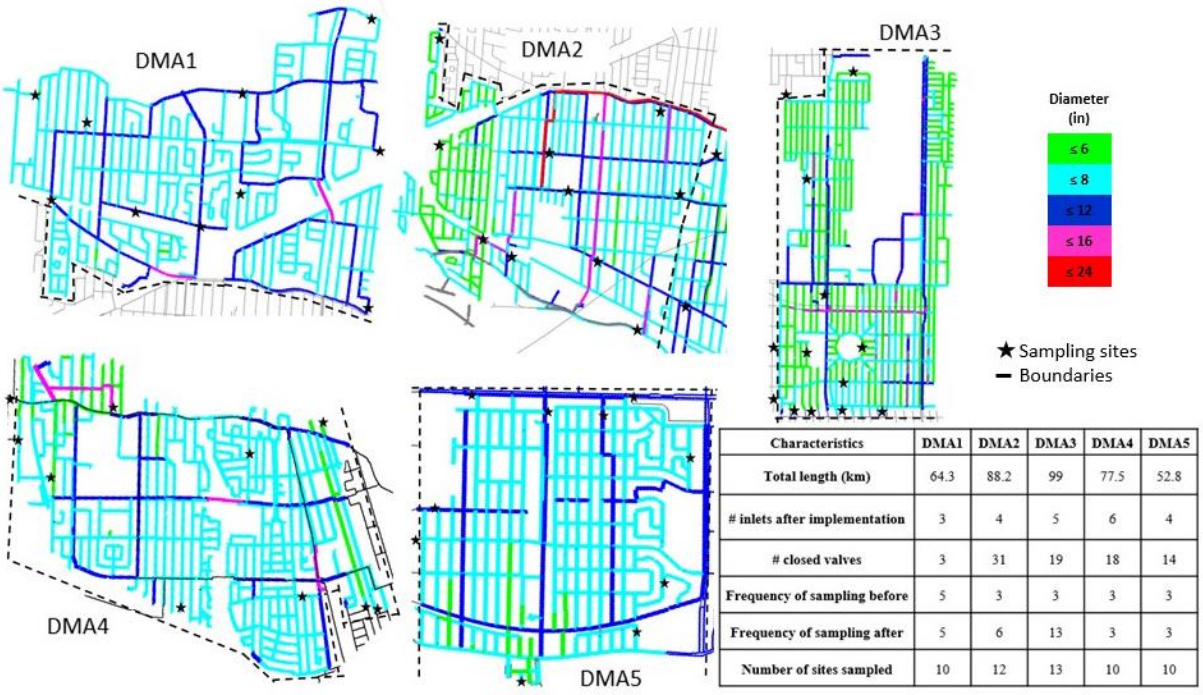
DMAs implementation while accounting for the changes in incoming water quality and distribution conditions; (3) verify if bacterial abundance and diversity varies between inlets, existing, and newly created dead-ends; (4) assess the relationships between water quality parameters and bacterial abundance and diversity; and (5) formulate recommendations on monitoring to assess potential risks of water quality deterioration after DMA implementation.

5.2 Materials and methods

5.2.1 Characteristics of the DMAs and choice of sampling sites

The DS of this study serves approximately 1.5 million people across nearly 4,000 km of pipes in Montreal (Canada). Two water treatment plants (WTPs) using surface water supply this system, and the treatment consists mainly of direct filtration, pre or post ozonation and chlorination. As part of the DS optimization program of this utility, five pilot studies for implementing DMAs were undertaken in 2012-2013. An overview of the characteristics of the DMAs, the sampling conditions and sampling sites are presented in Figure 5.1. Each DMA contained at least 10 sampling points, and these were sampled at least three times before and three times after the closing of valves to verify potential changes in water quality. DMA1 was sampled in September and October 2012, DMA2 was sampled in October and November 2012, DMA3 was sampled in June to September 2013, DMA4 was sampled in July and August 2013 and DMA5 was sampled in September and October 2013. Sampling was carried out preferably at the same time (morning peak) and same day of the week to ensure the same usage pattern in each DMA. The sampling sites were chosen to cover the potential changes caused by the DMA implementation based on hydraulic simulation results, the characteristics of the DMA, characteristics of the site and experience of the water utility staff.

Sampling locations were either inlets (to establish the baseline for water quality), existing and new created dead-ends after the closing of valves, outside DMA (near the boundaries), extremities (sites far away from the inlet), with changes in flow direction or water age variation, and compliance monitoring sampling points determined by the water utility. Sampled pipes in these sectors are made of cast iron or ductile iron (at few locations on DMAs 1 and 5) with internal pipe diameters varying from 4 to 14 inches.



Characteristics	DMA1	DMA2	DMA3	DMA4	DMA5
Total length (km)	64.3	88.2	99	77.5	52.8
# inlets after implementation	3	4	5	6	4
# closed valves	3	31	19	18	14
Frequency of sampling before	5	3	3	3	3
Frequency of sampling after	5	6	13	3	3
Number of sites sampled	10	12	13	10	10

Figure 5.1: Overview of the characteristics of DMAs and locations of sampling sites

5.2.2 Hydraulic network model

Hydraulic simulations were conducted using calibrated models provided by the water utility to assess the impact of DMAs on parameters that can influence the water quality such as the water residence time, water velocities and changes in flow direction. Simulations were performed on WaterGEMS V8i software with scenarios before and after DMAs implementation using average daily consumption and considering hourly variations patterns. Results have been taken from day 10 (using extended period simulation for 15 days), to ensure their stability and repeatability, at the minimum and maximum water demand conditions.

5.2.3 Sampling strategy and analytical methods

Water quality samples were taken at public buildings (only compliance monitoring sites) and outdoor household taps (both previously disinfected), after refreshing the pipe until the water temperature was constant to confirm that the water from the main pipe had reached the tap. All samples were taken following standard procedures, using sterile bottles (with sodium thiosulfate

for bacterial analyses), transported to the laboratory at 4°C and processed on the same day. General water quality parameters, such as temperature, pH, chlorine residuals, turbidity and conductivity were measured at the time of sampling with portable instruments according to standardized methods (American Public Health Association (APHA), *et al.*, 2005). Free and total chlorine residual measurements were conducted with the N,N-diethyl-p-phenylenediamine (DPD) colorimetric method using a DR/2010 spectrophotometer (HACH) according to Standard Methods 4500-Cl. DOC samples were filtered through pre-rinsed 0.45 µm hydrophilic polyethersulfone membranes (Supor®-450, PALL, USA), then analysis measurements were conducted on a laboratory total organic carbon analyzer (Sievers Instruments, Inc., GE, USA) according to Standard Methods 5310-C. Iron and manganese (dissolved and total for both parameters) were analyzed by ICP (Thermo Fisher iCAP 6000) according to Standard Methods (American Public Health Association (APHA), *et al.*, 2005). Particle counts were measured with Brightwell Dynamic Particle analyzer (DPA4100). Heterotrophic plate counts (HPC) were determined on R2A agar after incubation for seven days at 20°C according to method 9215-D (American Public Health Association (APHA), *et al.*, 2005). Total bacterial counts (TDCs) were determined by epifluorescence microscopy (Olympus, Tokyo, Japan) at 1000-fold magnification (Hobbie, *et al.*, 1977). Analysis were performed two or three times and averaged for each sample.

5.2.4 Planktonic bacterial community profiles from inlets, existing and created dead-ends

Samples were taken in DMAs 3, 4 and 5 from inlets, existing and created dead-ends before and after DMAs implementation at intervals of about four weeks. All samples were taken using sterile bottles, with sodium thiosulfate (1%). The samples were then transported to the laboratory at 4°C and processed on the same day. Samples were filtered through a 0.45 µm pore sized, 47 mm diameter, mixed cellulose ester membrane. For each DMA, samples were pooled at the time of filtration by mixing equal volumes of water from each sample (500 mL) by type of sampling point and condition (inlets, existing or created dead-ends and before/after implementations), totalizing 18 composite samples. All filtration equipment was sterilized by autoclaving prior to filtration. The filter was inserted into an extraction tube containing a garnet matrix and one 1/4-inch ceramic sphere (Lysing Matrix A, MP Biomedicals, Solon, USA) and cut in half. DNA from the obtained biomass was extracted directly on the filters using the bead-beating protocol adapted as previously

described (Yu & Mohn, 1999). Lysing buffer was added to each extraction tube prior to the bead-beating step performed on FastPrep-24 (MP Biomedicals), followed by ammonium acetate precipitation, and successive cold 70% ethanol washes. Extracted DNA was stored at -25°C until further analysis performed at Research and Testing Laboratory (Lubbock, TX, USA). The bacterial 16S rRNA gene was PCR-amplified for sequencing using a forward and reverse fusion primer as previously described (MacIntyre *et al.*, 2015). PCR products were sequenced using paired-end Illumina MiSeq sequencing (Illumina, Inc. San Diego, California) 2x300 flow cell at 10pM.

5.2.5 Raw HTS data accession number

The raw sequencing data were deposited in NCBI Sequence Read Archive (SRA) under accession number SRP076392.

5.2.6 Data analysis

To compare the water quality variables across sampling locations, mean plots and box-plots were generated so that trends could be identified. Nonparametric Wald-Wolfowitz Test was used to verify if the means (before and after values) at the inlets are statistically different ($\rho<0.05$). Associations between water quality parameters were achieved using Pearson correlations. Values for HPC and total bacterial cell counts were log-transformed to achieve a more normal distribution prior to investigate Pearson correlations. These analyses were achieved using Statistica 64 software v.13. Statistical significance is set to $\rho<0.05$ unless stated otherwise.

Raw sequencing reads were processed with the software Mothur v.1.34.4 (Kozich *et al.*, 2013). Quality filtering of reads was performed to remove low quality and chimeric sequences. Briefly, data were extracted from raw fastq files, and sequences containing ambiguous bases and/or longer than the expected fragment were discarded. Subsequently, sequences were aligned against the Silva reference alignment (Release 119) (Quast *et al.*, 2013). Potential chimeric sequences were identified using the UCHIME algorithm, and sequences not associated with bacteria were removed. Finally, sequences were clustered into operational taxonomic units (OTUs) with 97% identity. Rare OTUs identified at the 0.005% thresholds were removed (Bokulich *et al.*, 2013). Subsampling was performed to select sequences at random, corresponding to the sampling effort of the smallest library, to avoid downstream bias in statistical analysis due to varying sampling efforts. After quality filtering reads, eliminating rare sequences, and subsampling, the final OTU table was

assigned to different taxa levels using the RDP classifier (Release 11) (Wang *et al.*, 2007) with a minimum confidence threshold of 80% for bacteria and 50% for other taxonomic levels. Results of the UPGMA clustering, similarity profile analysis (α=0.05), and PCA were obtained using vegan, ade4, ape, and FactoMineR packages in R software (Paradis *et al.*, 2004; Dray & Dufour, 2007; Oksanen *et al.*, 2016).

5.3 Results and discussion

5.3.1 Changes in hydraulic parameter distribution

Changes in the distribution of water residence times and water velocities as well as changes in flow directions are expected following the implementation of DMAs. These changes of hydraulic regime can cause short-term and lasting water quality changes. The extent of these changes must be considered within the range of conditions corresponding to minimum and to maximum demand. Figures A-4.1 and A-4.2, presented in Appendix 4, summarize the distribution of changes for the five DMA studies for residence times and flow velocities respectively. Water age increased slightly (2 hours) in more than 50% of the nodes of DMA1 and minor variations (± 2 hours) were observed in other DMAs at maximum demand conditions. In DMA3, more important residence time variations (up to 10 hours) were observed at 55% of nodes. In some cases, such as DMAs 2 and 3, large increases in water residence times (≥ 20 hours) were observed in a small proportion of nodes (≈2%).

Limiting the sites with very high residence times is a priority for utilities as these are the locations where the maintenance of disinfectant residual and overall water quality is challenging. In the DMAs studied, the percentage of nodes with water residence time exceeding 50 hours ranged from 1.9-5.5% before to 1.9-6.6% after implementations. The number of nodes having very high residence times (>50h) increased by approximately 40% in some DMAs (2 and 3) and by about 10% in DMAs 4 and 5, while remaining stable in DMA1. DMA1 was almost already isolated before the implementation as only three valves were closed, while in the other DMAs between 14 and 31 valves were closed to form the boundaries (Figure 5.1). The changes in water velocities after the implementation of DMAs were minor (Figure A-4.2, Appendix 4) with no changes in 18-48% of the length of pipes after closing the valves at maximum demand conditions. These simulations show that the hydraulic changes in these pilot DMAs were minimal to moderate with a

redistribution of the parameters across the sector. Regardless, the number of dead-ends (>50 hours of residence time) did increase in 4 out of 5 DMAs, from 10% (89 to 98 nodes in DMA4) to 44% (142 to 205 nodes) in DMA2.

5.3.2 Water quality before and after the implementation of DMAs at inlets

Monitoring during an extended period in the DMAs must take into account significant water quality changes that are not related to the DMA implementation. Baseline values are needed to determine if observed changes in water quality in the DMAs can be attributed to the closing of valves, or if they are caused by changes in temperatures (different seasons), residual disinfectant or water treatment conditions. Water quality entering a DMA can vary over time reflecting seasonal changes in the source and operational adjustment of treatment and distribution. Water quality at inlets in each DMA constitutes the reference values during the monitoring period. Figure 5.2 shows the water parameters where some variations of water quality at DMA inlet points were observed. The most noticeable changes at inlets after DMAs implementation were observed for temperature which followed seasonal patterns: significant and gradual temperature decreases (3.3°C to 5.4°C) were observed at the DMAs monitored in the end of the summer and in the autumn (DMAs 1, 2, and 5) and a significant temperature increase (5.3°C) at DMA3 monitored in the end of spring and in summer. Temperature of water entering DMA4 monitored in summer increased only by 0.6°C. Free chlorine concentrations at inlets decreased significantly by 20% at DMA4. DOC varied significantly in the inlet points of DMAs 1, 2, and 3 reflecting seasonal DOC variations in the source water. After the implementation, particle counts decreased at all inlets (22-57%) but one (DMA3) where the largest increases in water velocity occurred. Significant different means were only observed in DMAs 3 and 5. These changes were not reflected by turbidity, which remained quite stable with rare spikes. Metal variations (iron and manganese) followed turbidity and/or particle counts trends. Iron and manganese mean concentrations were significantly different only at DMA2. The pH did not vary significantly in any of the DMAs. Bacterial abundance varied considerably at the DMA inlets, both at a given inlet and between inlets DMA implementation. Concentrations at inlets varied from 0.01 cfu/mL (minimum detection level) in DMAs 1 and 2 to 4.3 cfu/mL in DMA1. Significant decreases ($\rho<0.05$) in total bacterial cell counts at inlets were

observed in DMAs 1 and 3. Concentrations at inlets varied from 2.3E+02 cells/mL (DMA4) to 8.1E+04 in DMA3.

These significant variations in several water quality parameters at the inlet points of the DMAs show that the simple monitoring of water quality in the DMAs before and following implementation cannot be used to conclude on the impact of DMA implementation. The significant and gradual changes on incoming water temperature and the differences in chlorine residuals can impact bacterial quality.

5.3.3 Bacterial water quality changes with DMAs implementation

Bacterial water quality variations were investigated by measuring HPCs and total epifluorescence counts at various types of sites in the DMAs: inlets, newly created and existing dead-ends, points out of DMAs close to their new delineation, points with changes in flow direction or water residence time, extremities and compliance monitoring sites. Figure 5.3 present HPCs and total cell counts observed at DMAs grouped by the type of point to facilitate interpretation and guide the adjustment of post DMA monitoring programs. Overall, HPC and total counts values at inlets varied over 2.6 Log.

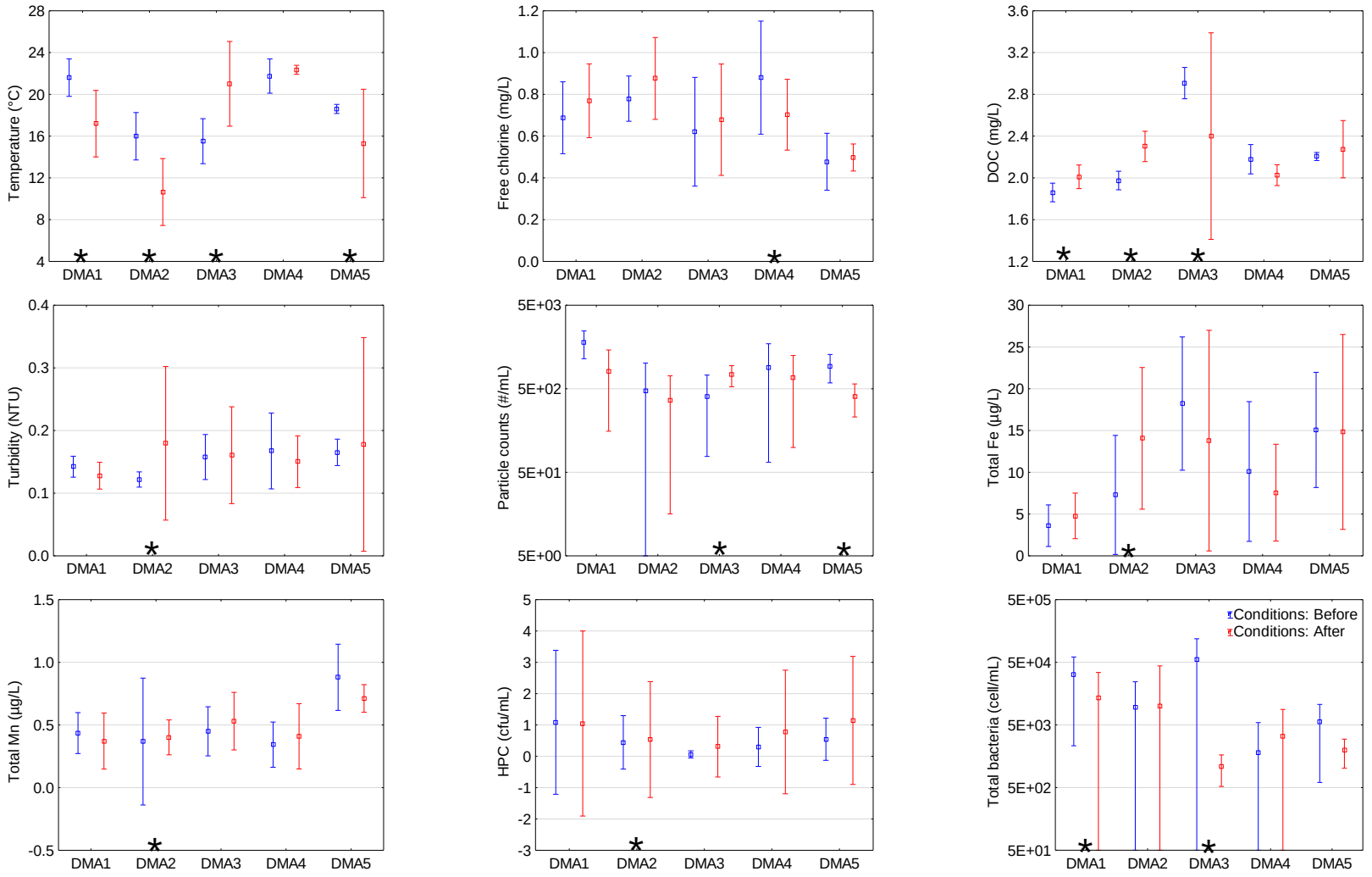


Figure 5.2: Mean plots of water quality parameters at inlets in each DMA. Blue lines represent measurements taken before the DMA implementation, and red ones those after the implementations. Whisker represent 2SD (standard deviation) and the squares mean values. Before and after groups are statistically different at $\rho<0.05$ by Wald-Wolfowitz Test (*)

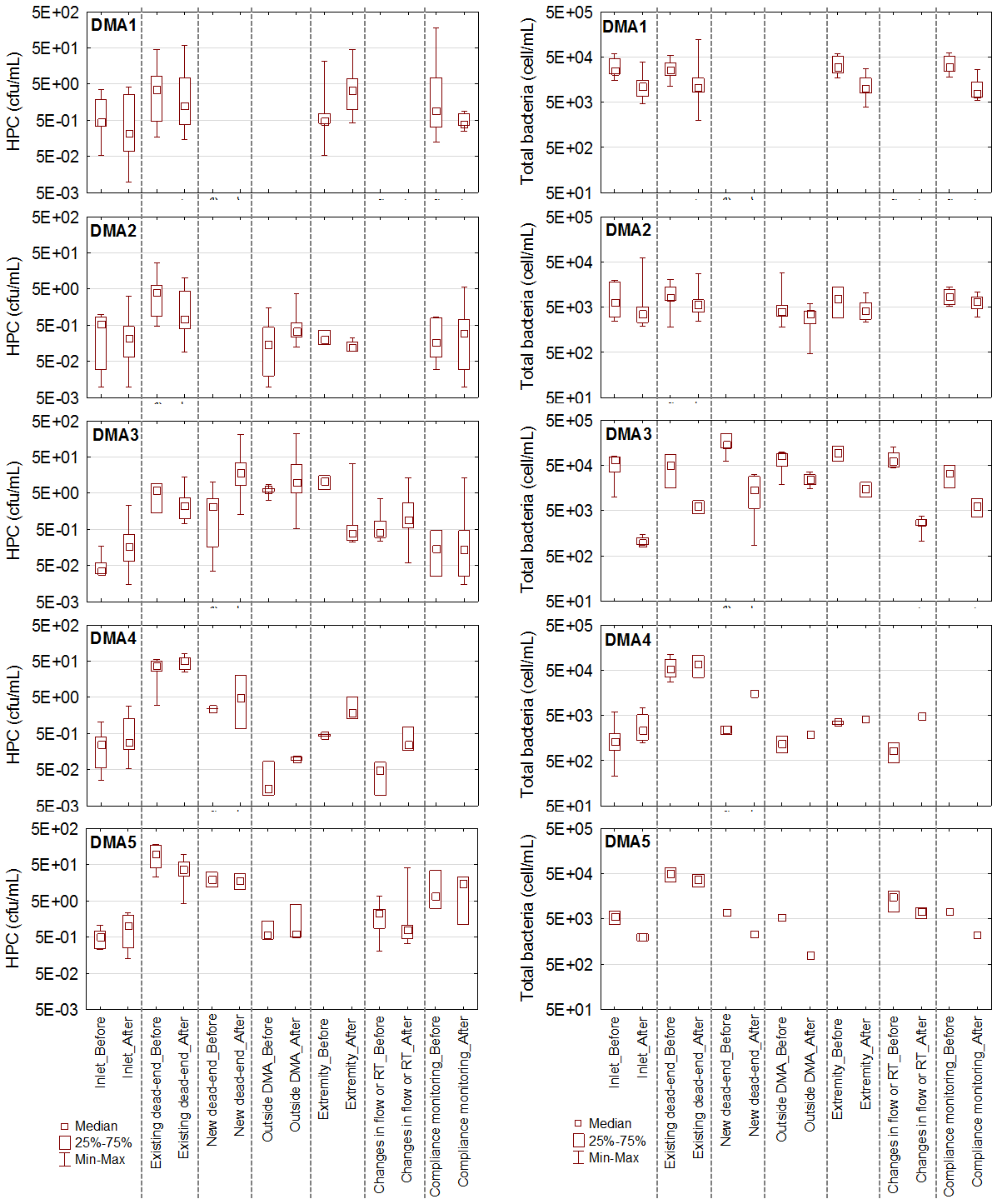


Figure 5.3: Box-and-whisker plots of heterotrophic plate count (HPC) and total bacterial counts across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values

Concentrations of HPC and total bacteria at existing dead-ends remained relatively stable, while some variations were observed in created dead-ends, outside sites, extremities and compliance monitored sites. In DMA3, higher HPCs were observed at the created dead-ends (average increase of 2.5E+01 cfu/mL), while a decrease of approximately 1 log was observed at extremity sites (changes in the water velocity at this site was not detected in the hydraulic simulation).

Changes in bacteria before and after DMAs implementation in inlets, existing and created dead-ends (DMAs 3, 4, and 5) were also investigated in terms of diversity by identifying microbial communities. The final OTU table consisted of 37,179 sequences and 1457 OTUs that were assigned to different taxa levels using the RDP classifier. A sample from an existing dead-end (DMA4) was removed of the dataset prior to subsampling due to the small number of sequences obtained from that sample. Sequences were classified into 19 bacterial phyla, 28 classes, 53 orders, 82 families, and 129 genera. The relative abundance of different phyla and subclasses of *Proteobacteria*, with the hierarchical clustering of the samples are presented in Figure 5.4. This analysis unveiled that response of microbial communities to DMA implementation was idiosyncratic. Indeed, the conditions prevailing at each DMA sector exerted a more significant impact on the clusterization of bacterial community profiles (Permanova, r^2=0.11, ρ=0.016). The majority of the samples (15/17) were composed by *Proteobacteria* (87-≈100%) with predominance of *Alphaproteobacteria* (52-95% in 14/17 samples). The predominance of *Alphaproteobacteria* in chlorinated DS is in accordance with other studies (McCoy & VanBriesen, 2012; Douterelo *et al.*, 2013; Lu *et al.*, 2013). Minor abundance of *Alphaproteobacteria* (42-55% in 4/6 samples) was observed mainly at inlets and at new created dead-end sites before implementation (4%). Higher concentrations of *Gammaproteobacteria* (35-47%), a Proteobacteria class known to have some potential pathogens, were observed at inlets in DMAs 3 and 5 where minor free chlorine concentrations were detected. Inlets presented similar community profiles, with exception of the inlet after in DMA5. This sample was more similar to profiles of new created and existing dead-ends sites. Changes in temperature, total cell counts, as well as the modifications in the flow caused by the closing of valves could be the factors influencing the communities in that sample. Changes in hydraulic conditions can affect bacterial structures and composition influencing the profiles of both water and biofilm communities (Douterelo, *et al.*, 2013). It is possible to note that some new created dead-ends after the implementation presented changes in the communities as expected. For example, new created dead-ends at DMA3 presented a completely different structure before/after

implementation, with the after sample becoming more similar to other existing dead-ends. The same occurred trend is somewhat observed with new created dead-end sample in DMA4.

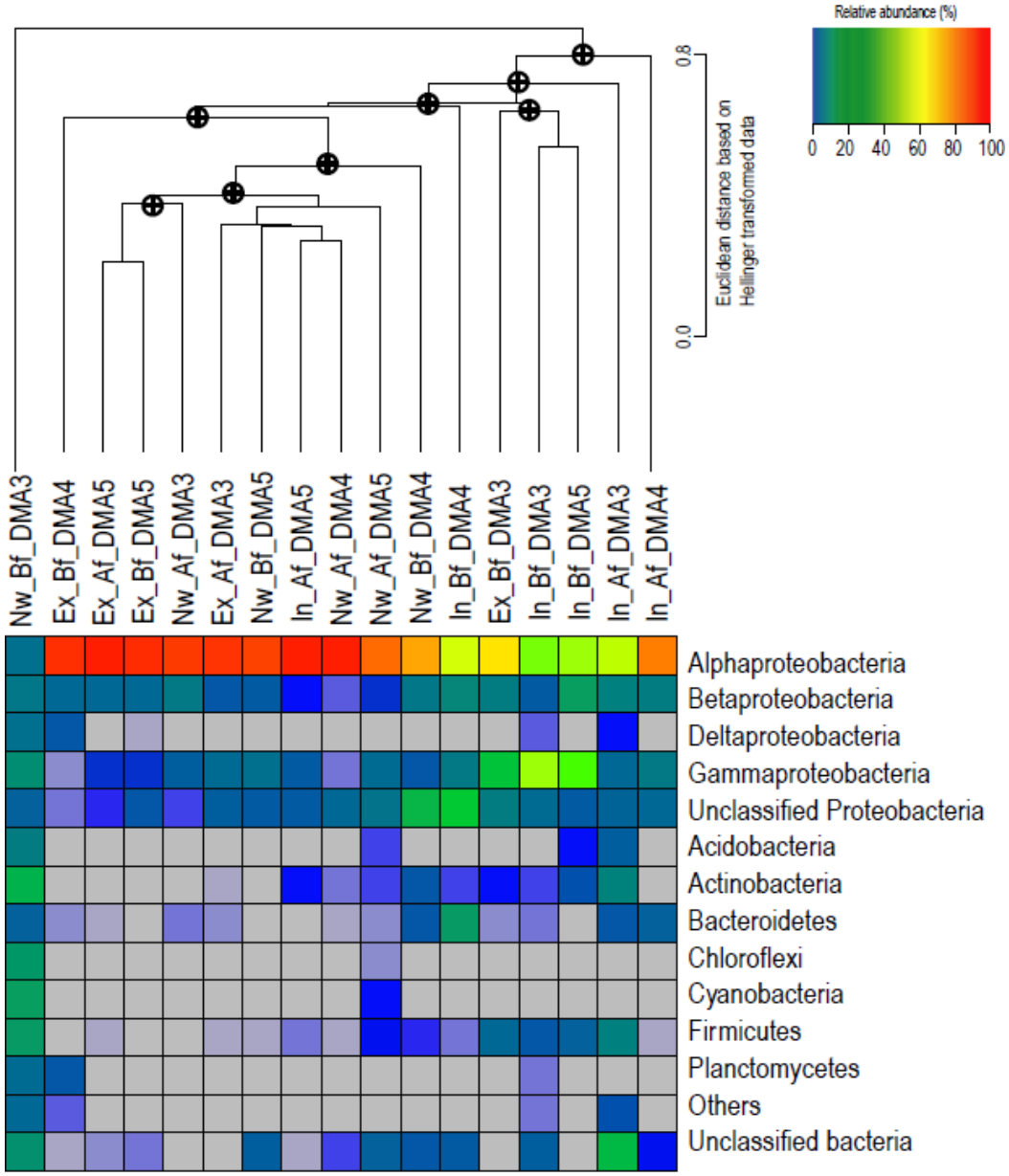


Figure 5.4: Heat map illustrates the relative abundance of different phyla and Proteobacteria classes in inlets (In), existing dead-ends (Ex), and new created dead-ends (Nw) samples before (Bf) and after (Af) implementations in DMAs 3, 4 and 5. Hierarchical clustering of samples is based on the similarity profile analysis of their bacterial community profiles (significant clusters at α=0.05). Samples with similar community structure cluster together, taking into account the relative abundance of each OTU

5.3.4 Influence of water quality parameters on bacterial variations and community structures across DMAs

Associations between bacterial abundance and water quality parameters measures at all types of sampling points (inlets, new and created dead-ends, extremities, flowing, reverse flow) in the five DMAs were first analyzed with simple Pearson correlations. Tables A-4.1 and A-4.2, presented in Appendix 4, show that HPCs were systematically correlated with water residence time and indicators of stagnation (iron, manganese, turbidity) and, as expected, inversely correlated with residual chlorine (r=0.49-0.83). Total cell counts were correlated with various parameters depending on the DMA considered, including pH, temperature, free chlorine, turbidity, DOC, and metals. DMAs 4 and 5, located further away from the water treatment plants, presented better correlations. Comparable correlations (r=-0.43 to -0.74) between HPCs and chlorine residuals have been reported in other systems (Carter, *et al.*, 2000; Zhang & DiGiano, 2002). Observed HPCs were quite lower than those reported in other Canadian distribution systems (Prévost, *et al.*, 1998; Francisque *et al.*, 2009) where a free chlorine below 0.3 mg/L was associated with HPC values exceeding 100 cfu/mL more frequently. Although a general trend of decreasing abundance with increasing residuals is clearly seen, wide range of HPCs and total counts can be encountered even at elevated concentrations of residual chlorine (>0.6 mg/L Cl_2) with HPCs (Figure 5.5a) and total counts (Figure 5.5b), as they range over 3.5 Log in various areas of the same DS. It is also notable that the correlation is not as strong depending on the DMA considered and that maintaining chlorine residuals is not effective to suppress culturable HPCs in DMA2. Higher bacteria levels were detected at lower chlorine residuals at DMAs 4 and 5, while at DMAs 1, 2, and 3 higher concentrations of total bacterial counts were found within a larger range of chlorine concentrations (Figure 5.5). These wide variations reflect both the variations in bacterial abundance at the inlet points and the intra DMA variations reflecting local conditions of water residence time (Figure 5.3). The lack of correlation in DMA2 suggests that not all of the bacteria variability can be explained by disinfectant residual. DMA2 was also the one where the lowest temperatures were observed, due to its monitoring taking place until the end of the autumn season. Although significant in most DMAs (Tables A-4.1 and A-4.2, Appendix 4), the correlations between residence time and bacterial abundance reveal large scatter and different trends between DMAs (Figures 5.5e and f). Correlations between HPCs and residence time have been reported previously

in full-scale (r=0.46) (Zhang & DiGiano, 2002), and at pilot-scale (Srinivasan *et al.*, 2008) chlorinated systems, while water residence time did not have a significant influence on bacterial levels (DAPI count) in unchlorinated DWDS (Sekar *et al.*, 2012). Figures 5.5e and 5.5f reveal that the influence of water residence times on abundance varies across the DMAs studied across this large distribution system and that inlet water quality variations are determining factor to consider to interpret local measurements. Previous studies also show a positive relationship between HPCs and pH (Carter, *et al.*, 2000; Zhang & DiGiano, 2002; Francisque, *et al.*, 2009). Negative correlations between bacteria and temperature were found in the DMAs where an increase in the inlet temperatures occurred after the closing of valves. Weak correlations between HPC occurrence and temperature (r=0.20-0.29) were previously reported in full-scale chlorinated systems (Carter, *et al.*, 2000; Zhang & DiGiano, 2002). Turbidity was correlated with HPCs and total counts in 4/5 DMAs (one marginally) in line with correlation (r=0.44) reported in a pilot-scale DS (Lehtola *et al.*, 2007). Particle counts were not generally correlated with bacterial abundance. Total and dissolved iron and manganese were well correlated with HPCs and to a lesser degree total bacteria. Increased concentrations of iron and manganese were found at locations with elevated residence time in small diameter unlined cast iron pipes which support higher levels of biomass and preclude the maintenance of any residual (Laurent, *et al.*, 2005b).

As water quality parameters are correlated, the impact of water quality was also investigated by principal component analysis (PCA) to identify variables that distinguish the different types of points before and after DMA implementation. Figure 5.6 shows that HPCs and total counts are inversely related to chlorine residuals while turbidity and metals are closely associated. Sites other than inlets and dead-ends are regrouped before and after DMA implementation, showing the determinant importance of the incoming water quality on water quality changes in the DMA. Water quality at these points of sampling is clearly influenced by the incoming water at the time of sampling (before versus after), more so than the changes brought by the isolation of the sector. Also notable is the fact that the newly formed dead-ends separate themselves and become closely associated with metals and turbidity, and, to a lesser degree, HPCs. Existing dead-ends before and after remain close with some dead-ends being more strongly associated with PC1.

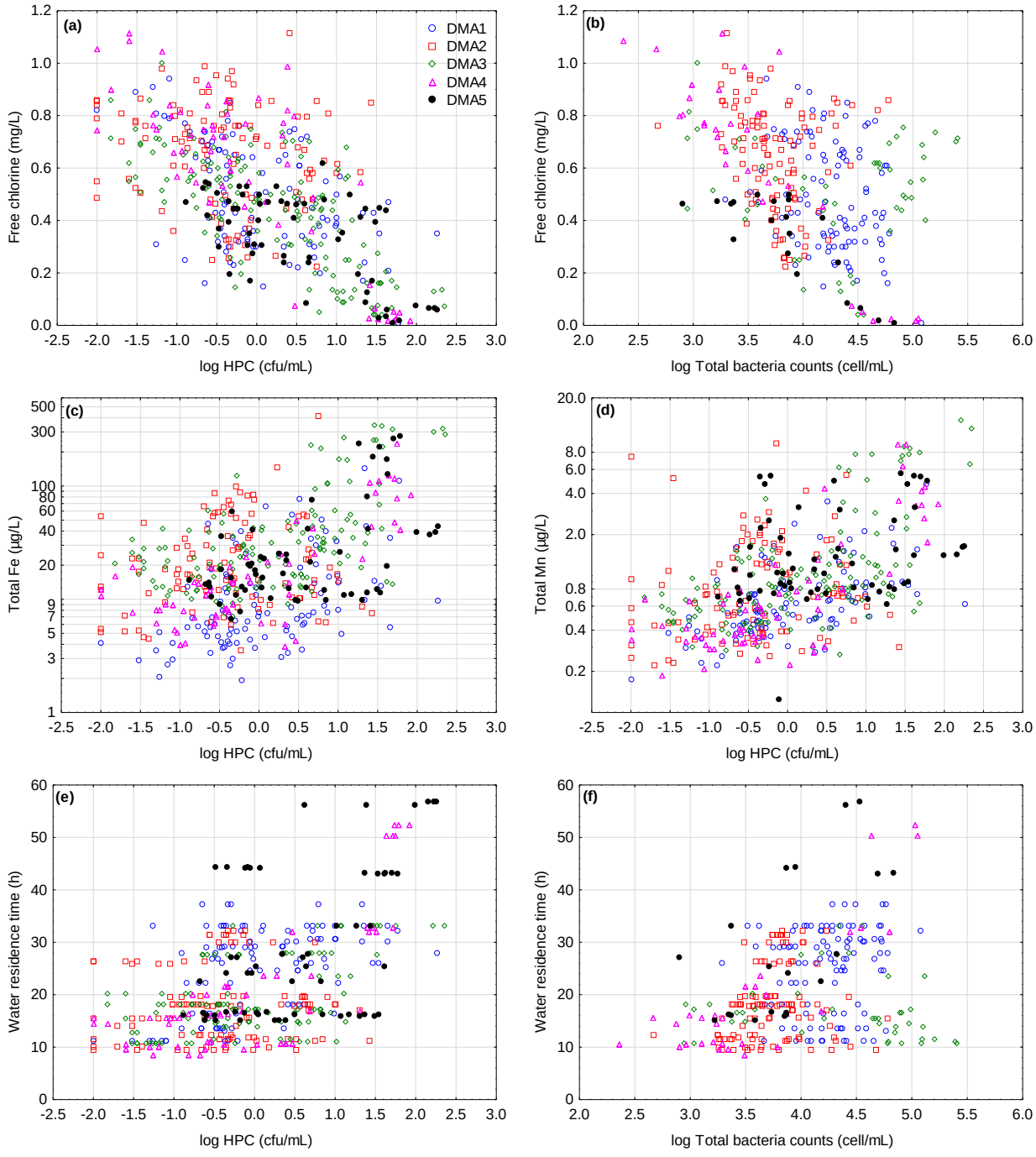


Figure 5.5: Relationship between the logarithm of HPC and total bacteria with the parameters that have most influenced bacterial changes for all dataset combined (DMAs 1-5)

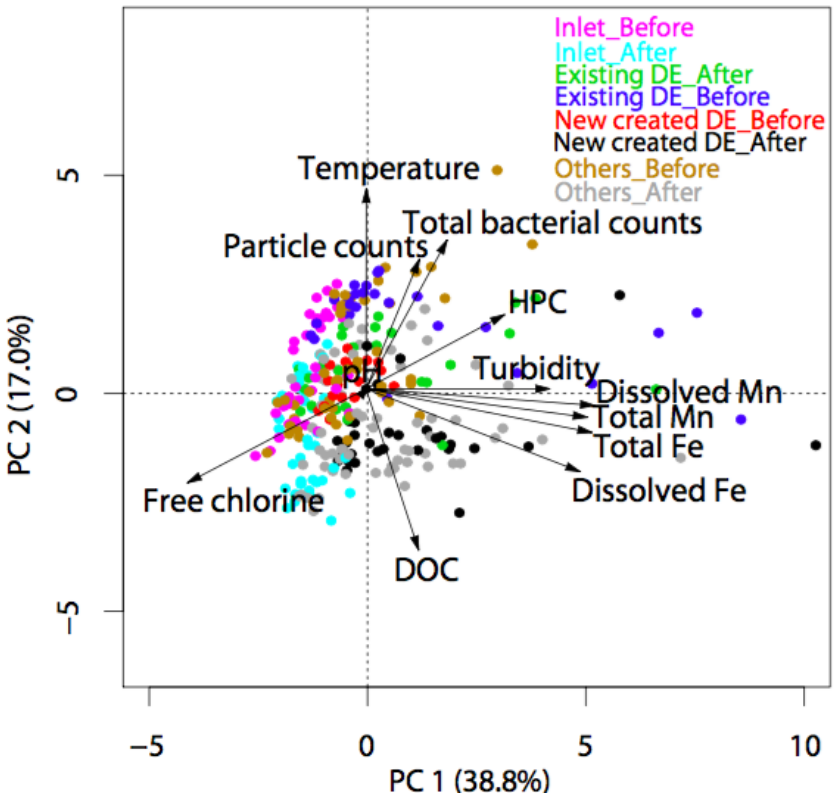


Figure 5.6: Dissimilarity in water quality parameters of different types of samples (inlets, existing and created dead-ends (DE), and other sites) before and after DMAs implementation

The relationship between the distribution of OTUs and water quality parameters was also assessed by means of PCA to explore dissimilarities between communities in the samples, as well as variables that could be influencing the taxonomic profile of bacterial communities (Figure 5.7). The first two axes (PC1 and PC2) explain 32% of the variability observed in the bacterial communities across the inlet, created and existing dead-end samples. In this figure, arrows indicate the direction of change in the respective water quality parameter. The length of the arrow is proportional to the loading of water quality parameter on the ordination of bacterial community structure. Significant parameters that have higher influence in the microbial communities include total bacteria counts (r^2=0.71, ρ<0.05), free chlorine (r^2=0.58, ρ<0.05), total manganese (r^2=0.56, ρ<0.05), total iron (r^2=0.48, ρ<0.05), HPC (r^2=0.35, ρ<0.1), DOC (r^2=0.32, ρ<0.1), and turbidity (r^2=0.30, ρ<0.1). An interesting trend is noted as diversity is determined by two sets of parameters: culturability shown by the factors related to HPCs (Mn, Fe, turbidity, and chlorine) and total

bacteria related to organic matter content (DOC). Conditions present at high water residence time that lead to increased HPC select for different taxonomic compositions.

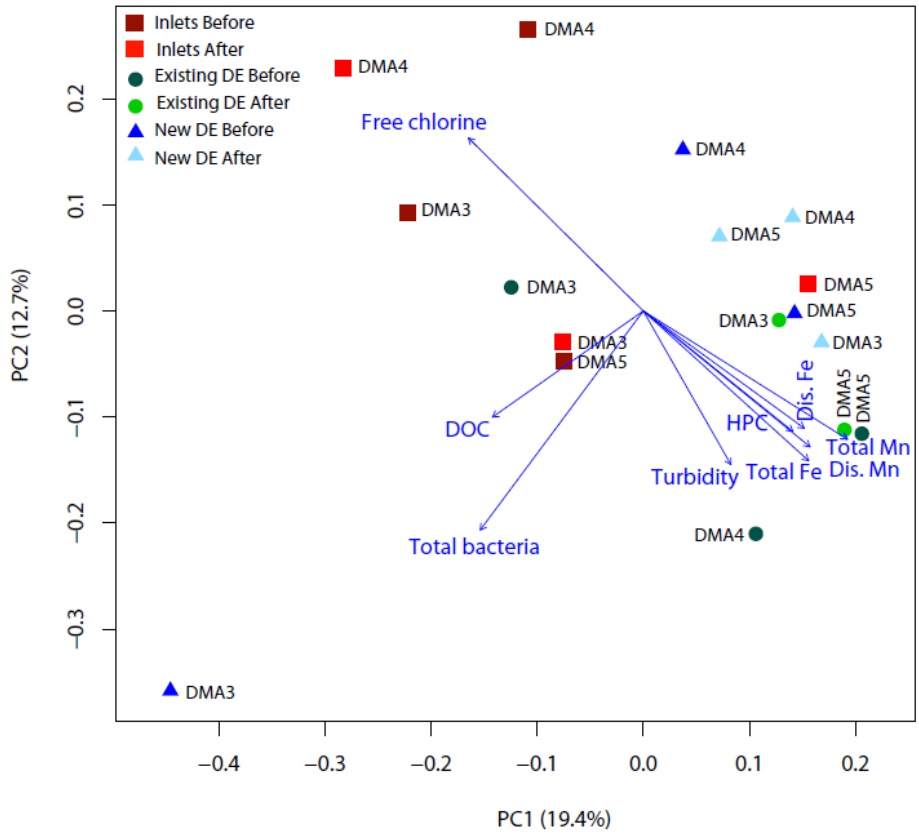


Figure 5.7: Ordination plot of principal component analysis (PCA) showing community dissimilarities in inlets, existing and new dead-ends (DE) samples before and after implementations for DMAs 3, 4 and 5. The influence of environmental variables (significant variables at $\rho<0.1$) are presented blue vectors. Axes PC1 and PC2 explained 32% of the variability in the community dissimilarities data

During the pilot DMAs, pressure was not regulated. The use of pressure management will reduce leaks and is likely to increase the residence times as well as lower velocities in some pipes. Consequently, the changes in water quality observed should be considered as a baseline.

5.4 Conclusions

Full-scale field monitoring was conducted during the temporary implementation of five pilot DMAs to document water quality (chlorine residuals, pH, conductivity, particles, turbidity, metals, etc.) and to investigate bacterial abundance and community structures inside and outside of DMAs boundaries. This investigation was carried out using an enhanced water monitoring sampling plan that takes into account the hydraulic characteristics of the sampling points (dead-ends, extremities, entry points, compliance reference points, changes in flow direction) with an effort to compare them in terms of hydraulic conditions before and after the isolation of the sector.

Overall, the hydraulic changes caused by the isolation during the temporary implementation of DMAs were minimal and their extent depended on the configuration of the DMA. Nevertheless, the simulations showed that the number of locations with higher water residence time (>50 hours) increased significantly. At these points of elevated residence time, it was demonstrated that bacterial abundance and composition reflected both water quality at inlets and hydraulic characteristics in the DMAs. Dead-ends were clearly critical water quality points for HPCs, and to lesser extent total counts. Levels observed in newly formed dead-ends were similar to those observed in existing dead-ends. In some DMAs, the impact of higher water residence time or stagnation on bacterial abundance in sites such as created dead-ends, outside sites, and extremities were observed, while water quality and bacterial abundance mostly remained constant at existing dead-ends.

The analysis of bacterial community structures revealed that:

- Dissimilarities among communities in the samples were partly explained by water quality parameters. The parameters that most influenced the communities were total bacterial counts, free chlorine, and metals (iron and manganese);
- The response of bacterial community structure to the implementation of DMAs is idiosyncratic, depending on hydraulic and physicochemical features of each DMA.

Our results show that DMAs should be designed to minimize, as much as feasible, the number of sites with anticipated elevated water residence time. Utilities implementing DMAs can quantify the risk of potential water quality issues associated with the closure of sectors by combining: (1) hydraulic modeling to quantify the number of sites with elevated residence time; and (2) documenting the quality at existing high risk sites by targeted monitoring. With this information,

the extent of potential water quality problems could be estimated and the best solutions to manage potential water quality problems be identified. As dead-ends represent critical points for water quality in a DS, utilities should understand and anticipate microbial quality in their existing and newly created dead-ends.

Further research is needed to understand the evolution of microbial quality in dead-ends and its sanitary significance.

5.5 Acknowledgements

This study was supported by the partners of the NSERC Industrial Chair on Drinking Water. The authors thank the Chair staff, especially Mireille Blais, Yves Fontaine, Jacinthe Mailly and Julie Philibert. The authors would also to thank the utility technical personnel of the City of Montreal for providing hydraulic model and support, and Bentley systems for providing academic access to the utility model. Eric Déziel holds the Canada Research Chair in Socio-Microbiology.

CHAPTER 6 ARTICLE 3 – IDENTIFICATION OF FACTORS AFFECTING BACTERIAL ABUNDANCE AND COMMUNITY STRUCTURES IN A FULL-SCALE DRINKING WATER DISTRIBUTION SYSTEM

This chapter reports the results from a detailed systemic investigation on bacterial abundance and community structures across different sub-systems in a full-scale drinking water distribution system including water quality parameters. We compared the bacterial profiles from water leaving two water treatment plants, from different locations in five distribution system sectors before and after DMAs implementation and from ten taps in a hospital using high-throughput sequencing of PCR-amplified 16S rRNA genes. By pooling samples from the distribution, a more global view of bacterial community structures throughout the system can be taken. In this case, all data before and after DMAs implementation of each sector were considered together. This chapter is a paper submitted to *Applied and Environmental Microbiology*. Supplementary information is presented in Appendix 5.

IDENTIFICATION OF FACTORS AFFECTING BACTERIAL ABUNDANCE AND COMMUNITY STRUCTURES IN A FULL-SCALE DRINKING WATER DISTRIBUTION SYSTEM

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Running Head: Bacterial communities in water distribution systems

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ABSTRACT

The taxonomic structure of bacterial communities in a full-scale water distribution system in North America was assessed via high-throughput sequencing of PCR-amplified 16S rRNA genes. We aimed to assess whether bacterial communities detected in treated water determine community structures in main pipes and premise plumbing. Our results indicate that bacterial community compositions differ between treated, distributed water, and premise plumbing. While *Proteobacteria* (60%), *Planctomycetes* (20%), and *Bacteroidetes* (10%) were the most abundant phyla in treated water, *Proteobacteria* largely dominated distribution system sites (98%) and taps (91%). Nevertheless, profiles of *Proteobacteria* diverged between distributed and tap waters. *Alphaproteobacteria* was the most dominant class in distributed water (92% vs. 65% in tap waters) and *Betaproteobacteria* was most abundant in tap waters (18% vs. 2% in distribution system). Hierarchical clustering of samples based on the similarity of bacterial community profiles resulted in four clusters: one from each water treatment plant, one from the distribution system, and one from taps. Unconstrained ordination of the ribotyping profiles was partly explained by differences in chlorine residual concentrations, total bacterial counts and water residence times. Ribotypes found within the entire system were identified, including those restricted to treated water, distributed water, or premise plumbing. This is the first study to apply high-throughput sequencing of 16S rRNA genes for a complete investigation across a full drinking water distribution system including treated water, different sectors in the distribution system, taps from premise plumbing, and water quality parameters. Overall, this study provides insight into the determinant influence of the water distribution system, the presence of a residual disinfectant on bacterial communities in the water, and hydraulic conditions, thus furthering our understanding of distribution bacteriological water quality in pipes and enabling better management of bacterial potential contaminants in drinking water distribution systems and premise plumbing.

6.1 Introduction

The use of residual disinfectants is a common practice for limiting microbiological contaminants in drinking water distribution systems (DWDS) to deliver safe water to customers of North America. Nevertheless, due to complex interactions between chemical, physical, and operational parameters, bacterial regrowth and proliferation of resistant pathogens in distribution systems can occur even in the presence of a residual disinfectant (Zhang & DiGiano, 2002; Berry, *et al.*, 2006; Liu, *et al.*, 2013). Amplification of bacteria occurs throughout DWDS and significant differences in bacterial growth can be observed in premise plumbing due to unavoidable stagnation, low levels of disinfectant residual, and pipe material (Manuel *et al.*, 2010; Prévost, *et al.*, 2014; Proctor & Hammes, 2015). Opportunistic premise plumbing pathogens (OPPPs) are microbial inhabitants of drinking water, and the primary cause of waterborne disease outbreaks in developed countries (Hilborn *et al.*, 2013; Water Research Foundation (WRF), *et al.*, 2013). The presence of OPPPs and their potential health effects are especially worrisome in hospitals, since these bacteria have been linked to cases of waterborne infections among susceptible patient populations (Perola *et al.*, 2002; Lucero *et al.*, 2011; Williams *et al.*, 2013).

The development of more advanced techniques, such as high-throughput sequencing (HTS) procedures, has overcome the limitations of more conventional methods, such as culture-based and molecular fingerprinting (Forney, *et al.*, 2004), and enables high-resolution characterization of microbial populations in DWDS. Several groups have investigated bacterial abundances using HTS techniques in actual municipal DWDS with or without disinfectants, providing a better understanding of the planktonic microbial community structure in different locations throughout the systems (Hwang *et al.*, 2012; Pinto, *et al.*, 2012; Lautenschlager, *et al.*, 2013; Holinger *et al.*, 2014; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015). In several studies, the planktonic bacterial composition across full-scale DWDS remains relatively stable over time with the exception of minor changes at sites with longer water residence times (Hwang, *et al.*, 2012; Pinto, *et al.*, 2012; Lautenschlager, *et al.*, 2013; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015). In regard to premise plumbing, a recent study highlighted the importance of water treatment and distribution management choices in defining the composition of premise plumbing microbial communities (Ji *et al.*, 2015). Some of the research previously mentioned have revealed minimal changes in bacterial composition across DWDS, even in systems

using residual disinfectants, in locations with high residence times where a significant degradation of water quality is usually observed. Moreover, certain of these studies highlight the similarities of communities across the system with observations based on the phylum taxonomic level.

To the best of our knowledge, no existing research has used HTS of 16S rRNA genes to analyse all sub-systems of a full-scale DWDS, namely: treated water, different distribution system sectors, and taps from premise plumbing sites. In this study, we investigated the diversity and relative abundance of the planktonic bacterial communities in a chlorinated full-scale DWDS in Canada. We compared the bacterial profiles from water leaving two treatment plants, from different locations in five distribution system sectors and from ten taps in a hospital. Our main objectives were to answer the following questions: (i) Does bacterial community composition vary between treated water, distributed water, and premise plumbing? (ii) Which microbial taxa are shared between these sub-systems? (iii) Can cosmopolitan and endemic species be identified across the sub-systems? (iv) Lastly, do water quality parameters influence the structure and diversity of microbial communities? Overall, this study delivers a better understanding into the factors influencing bacterial communities in the water after the treatment plant across the distribution system pipeline and premise plumbing, thus enabling better management of bacterial contaminants in DWDS.

6.2 Materials and methods

6.2.1 Water sampling

A total of 28 water samples were taken from 2012 to 2014 from two water treatment plants (4), five sectors in the distribution system (10), and ten taps in a hospital (10) in Canada. Figure 6.1 presents the schematic diagram of the DWDS used for this study. This system serves approximately 1.5 milion people across nearly 4,000 km of pipes. Treated water samples provided by two treatment plants (TWA and TWB, both supplied by surface water) were collected four times from each plant after chlorination. The samples were collected at intervals of approximately three weeks between each sampling (during the summer season of 2014). Water treatment at both facilities consisted mainly of flocculation, filtration, ozonation and chlorination. Sampling in the distribution system was performed twice from five sectors (DS1 through DS5) at intervals of about four weeks. DS1 had a total length of 64.3 km and was sampled at ten different sites (two inlets, two dead-ends,

and six sites along the sector). DS2 was 88.2 km long and sampled at 12 different sites (three inlets, four dead-ends, and five sites along the sector). DS3 had a length of 99 km and was sampled at 13 sites (two inlets, six dead-ends, and five sites along the sector). DS4 was 77.5 km long and sampled at ten sites (three inlets, four dead-ends, and three sites along the sector). DS5 was 52.8 km long and sampled at ten sites (two inlets, four dead-ends, and four sites along the sector).

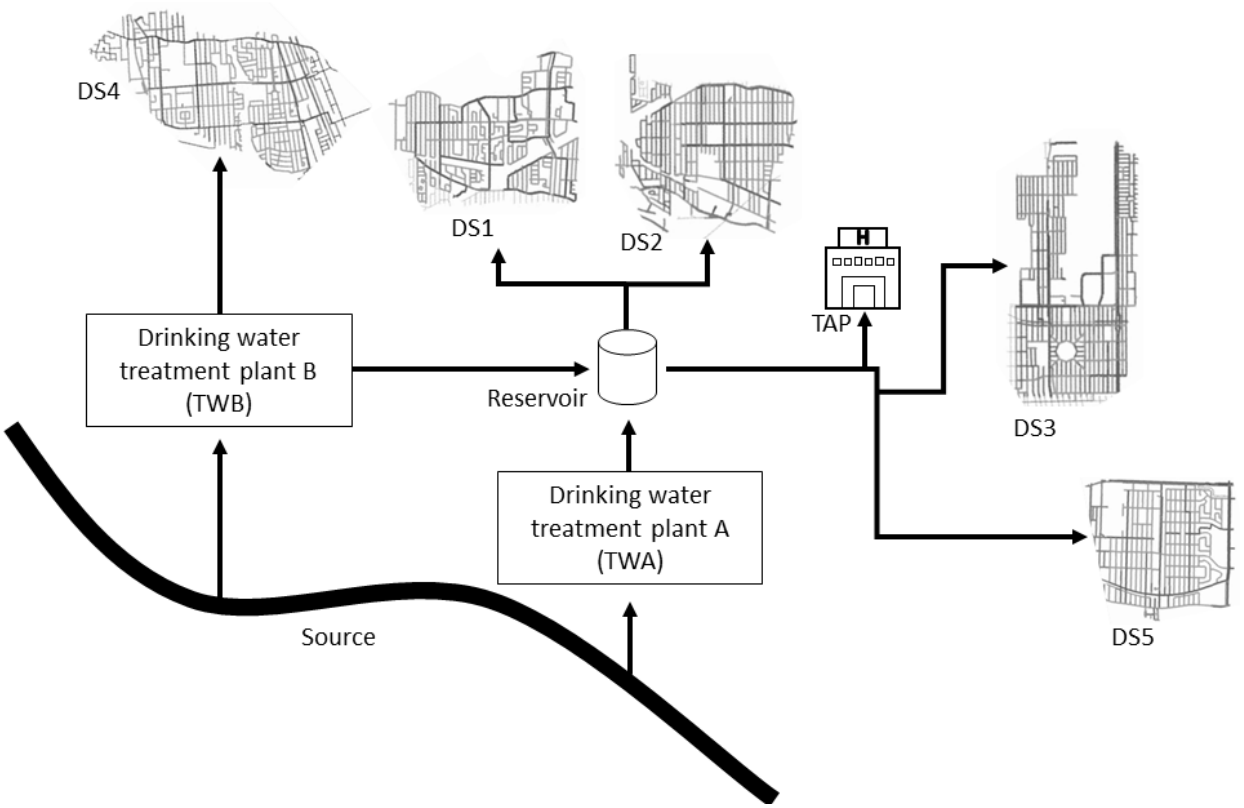

Figure 6.1: Schematic diagram of the drinking water distribution system sampled illustrating the sub-systems: treated water (TW), distribution system (DS), and premise plumbing (TAP)

The pipes sampled in these sectors were made of cast iron or ductile iron (at a few locations on DS1 and DS5) with diameters ranging between 4 and 14 inches. The water from the distribution system was sampled at outdoor household taps after refreshing the pipe until the water temperature was constant, confirming that the water from the main pipe had reached the tap. DS1 and DS2 were sampled in the fall of 2012; DS3 and DS4, in the summer of 2013; and DS5, in the fall of 2013. Samples from premise plumbing (copper material) were taken from standard and pedal taps, from four different floors comprising five hospital departments (see Table A-5.1 in the Appendix 5).

Sampling was performed once (summer of 2013) at each of ten taps (TAP1 through TAP10) collecting the first flush cold water after one-night of stagnation. All samples were taken using sterile bottles, with sodium thiosulfate (1%) to neutralize the activity of disinfectants. The samples were then transported to the laboratory at 4°C and processed on the same day.

6.2.2 Water quality analysis

All water samples were analyzed for free chlorine on site using the *N*,*N*-diethyl-*p*-phenylenediamine method and a DR/2010 spectrophotometer (HACH). Total cell counts were determined by fluorescence microscopy (Olympus, Tokyo, Japan). All analyses were performed in triplicate for each water sample, and the average of the three replicates was calculated. Hydraulic residence time at each location was determined using calibrated models by WaterGEMS V8i software. For tap water samples, viable and total cell counts were determined using a LIVE/DEAD BacLight Bacterial Viability Kit (Molecular Probes, Eugene, USA) (Boulos *et al.*, 1999). Briefly, 1 mL of the sample or dilution thereof in 0.85% sterile saline solution was mixed with 3 µL of stain (propidium iodide and SYTO9), incubated in the dark for 15 min and filtered on a black 0.2 µm pore diameter, 25 mm diameter polycarbonate filter (Millipore, Bedford, USA). Enumeration was done at 1000-fold magnification, with an epifluorescence microscope (Olympus, Tokyo, Japan). Total copper concentrations were measured by ICP-MS following the EPA 200.8 method with prior acid digestion in 0.5% HNO_3 for 24 h (United States Environmental Protection Agency (USEPA), 1994).

6.2.3 DNA extraction and bacterial 16S rRNA gene PCR-amplification and sequencing

Samples were filtered through a 0.45 µm pore sized, 47 mm diameter, mixed cellulose ester membrane. Specifically, 10 L of treated water from each plant, and 500 mL of each sample from distribution system and the tap waters were individually filtered. For each distribution system sector, the samples acquired for the different locations were pooled at the time of filtration by mixing equal volumes of water from each sample (500 mL). All filtration equipment was sterilized by autoclaving prior to filtration. The filter was inserted into an extraction tube containing a garnet matrix and one 1/4-inch ceramic sphere (Lysing Matrix A, MP Biomedicals, Solon, USA) and cut in half. DNA from the obtained biomass was extracted directly on the filters using the bead-beating

protocol adapted as previously described (Yu & Mohn, 1999). Lysing buffer was added to each extraction tube prior to the bead-beating step performed on FastPrep-24 (MP Biomedicals), followed by ammonium acetate precipitation, and successive cold 70% ethanol washes. Extracted DNA was stored at -25°C until further analysis performed at Research and Testing Laboratory (Lubbock, TX, USA). The bacterial 16S rRNA gene was PCR-amplified for sequencing using a forward and reverse fusion primer as previously described (MacIntyre, *et al.*, 2015). PCR products were sequenced using paired-end Illumina MiSeq sequencing (Illumina, Inc. San Diego, California) 2x300 flow cell at 10pM.

6.2.4 Data analysis

Raw sequencing reads were processed with the software Mothur v.1.34.4 (Kozich, *et al.*, 2013). Quality filtering of reads was performed to remove low quality and chimeric sequences. Briefly, data were extracted from raw fastq files, and sequences containing ambiguous bases and/or longer than the expected fragment were discarded. Subsequently, sequences were aligned against the Silva reference alignment (Release 119) (Quast, *et al.*, 2013). Potential chimeric sequences detected identified using the UCHIME algorithm, and sequences not associated with bacteria were removed. Finally, 589,411 sequences were clustered into 141,049 operational taxonomic units (OTUs) with 97% identity. Rare OTUs identified at the 0.005% thresholds were removed (Bokulich, *et al.*, 2013). A sample from treatment plant A (TWA-4) was removed prior to subsampling due to the small number of sequences obtained from that sample. Subsampling was performed to select 1013 sequences at random, corresponding to the sampling effort of the smallest library, to avoid downstream bias in statistical analysis due to varying sampling efforts. After quality filtering reads, eliminating rare sequences, and subsampling, the final OTU table consisted of 27,351 sequences and 762 OTUs that were assigned to different taxa levels using the RDP classifier. Venn diagrams were implemented to verify the shared OTUs between sample groups. Mothur was also used to calculate richness estimators and diversity indices. The taxonomic assignation of OTU was based on the RDP classifier (Release 11) (Wang, *et al.*, 2007) with a minimum confidence threshold of 80% for bacteria and 50% for other taxonomic levels. Results of the UPGMA clustering, similarity profile analysis (α=0.05), PCA and indicator species analysis (Indval) were analyzed using vegan, ade4, ape, and indicspecies packages in R software (Paradis, *et al.*, 2004; Dray & Dufour, 2007; De Cáceres & Legendre, 2009; Oksanen, *et al.*, 2016). Relationships between water quality

variables and PCA coordinates were investigated using non-parametric Spearman's rank correlation coefficients by STATISTICA 64.

6.2.5 Raw HTS data accession number

The raw sequencing data were deposited in NCBI Sequence Read Archive (SRA) under accession number SRP071223.

6.3 Results

6.3.1 Amplification of total bacterial counts and decrease in disinfectant residual concentration through the DWDS

Figure 6.2 shows that free chlorine concentration decreased when the water residence time increased from water treatment plants (TW samples) to all sectors of the distribution system (DS samples) and taps from premise plumbing (TAP samples) (-0.68, $p<0.05$). The concentration of disinfectant residual ranged from 0.8 to 1.8 mg/L in TW samples, 0.01 to 1.1 mg/L in DS samples, and 0 to 0.10 mg/L in TAP samples from premise plumbing (which was the lowest). Total bacterial counts ranged in order of magnitude from 10^3 to 10^4 cells/mL in TW samples, 10^2 to 10^5 cells/mL in DS samples, and 10^4 to 10^5 cells/mL in TAP samples. Total bacterial counts increased while chlorine residuals decreased (-0.51, $p<0.05$), mostly at stagnation sites and TAP samples. Large variations of disinfectant residual concentrations, water residence times and total bacterial counts were observed over the five sectors in DS samples. This is most likely because these samples consisted of a combination of different sampling points such as inlets (high disinfectant residuals, low levels of water residence time and total bacteria), dead-ends (low disinfectant residuals and higher levels of water residence time and total bacteria), as well as points along the sectors encompassing intermediate values.

6.3.2 Bacterial community diversity and composition

Diversity analyses, including richness estimators and diversity indices, were used to assess the level of richness and evenness of the sequences using a 3% dissimilarity cut-off (see Table A-5.2 in the Appendix 5). The coverage of the sequence libraries was high, ranging from 97 to 98%, confirming that the core communities were covered in all samples. Based on the Shannon diversity

index, species richness of bacterial community in the distribution system appeared lower when compared to TW and TAP communities. However, ACE and Chao richness estimators indicated that bacterial communities in the DS samples were somewhat richer than TW and TAP samples.

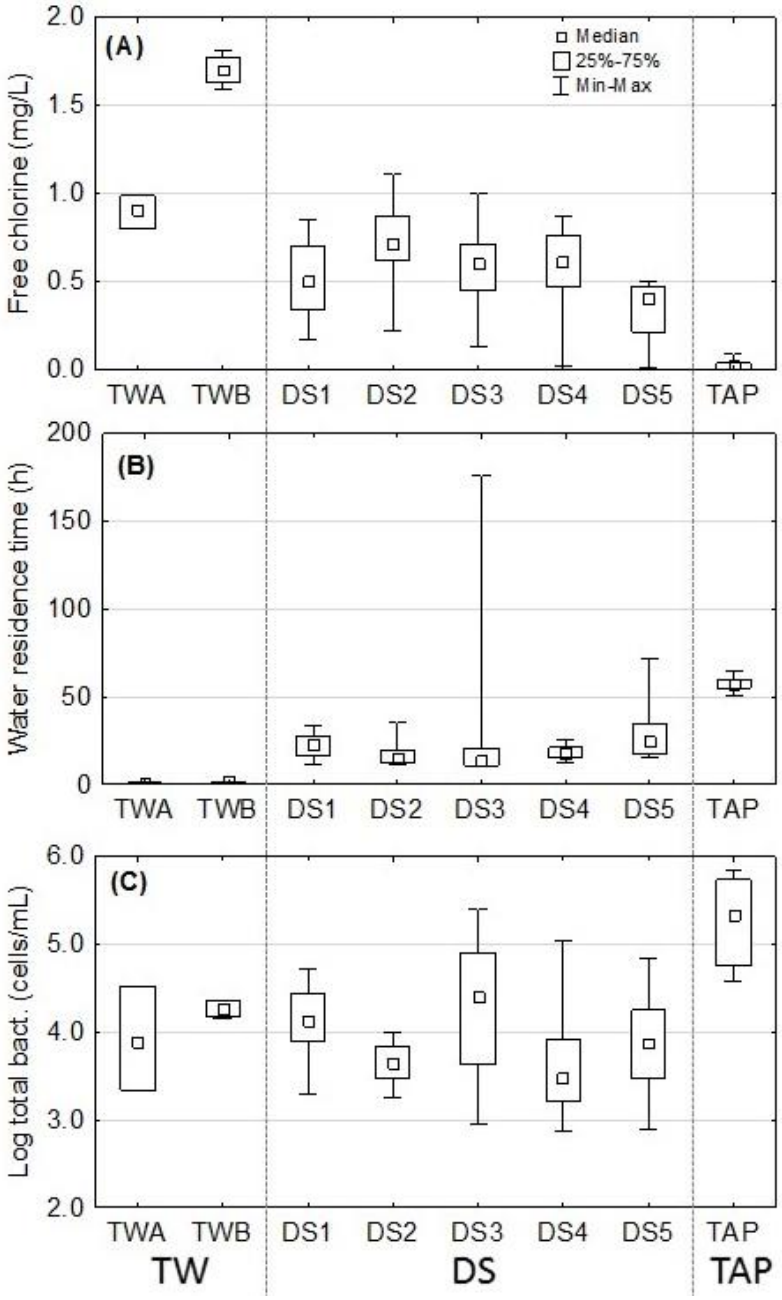

Figure 6.2: Box-plot showing the water quality across the sub-systems (treated water (TW), distribution system (DS), and premise plumbing (TAP)): (A) free chlorine, (B) water residence time, (C) log of total bacterial counts

Sequences were classified into 18 bacterial phyla, 37 classes, 64 orders, 95 families, and 149 genera. The relative abundance of different phyla and subclasses of *Proteobacteria*, as well as the hierarchical clustering of the samples are shown in Figure 6.3. The bacterial community structure changed noticeably among the different sub-systems resulting in four clear clusters: one from each water treatment plant, one from distribution systems (9/10 samples) and one from taps of premise plumbing (8/10 samples).

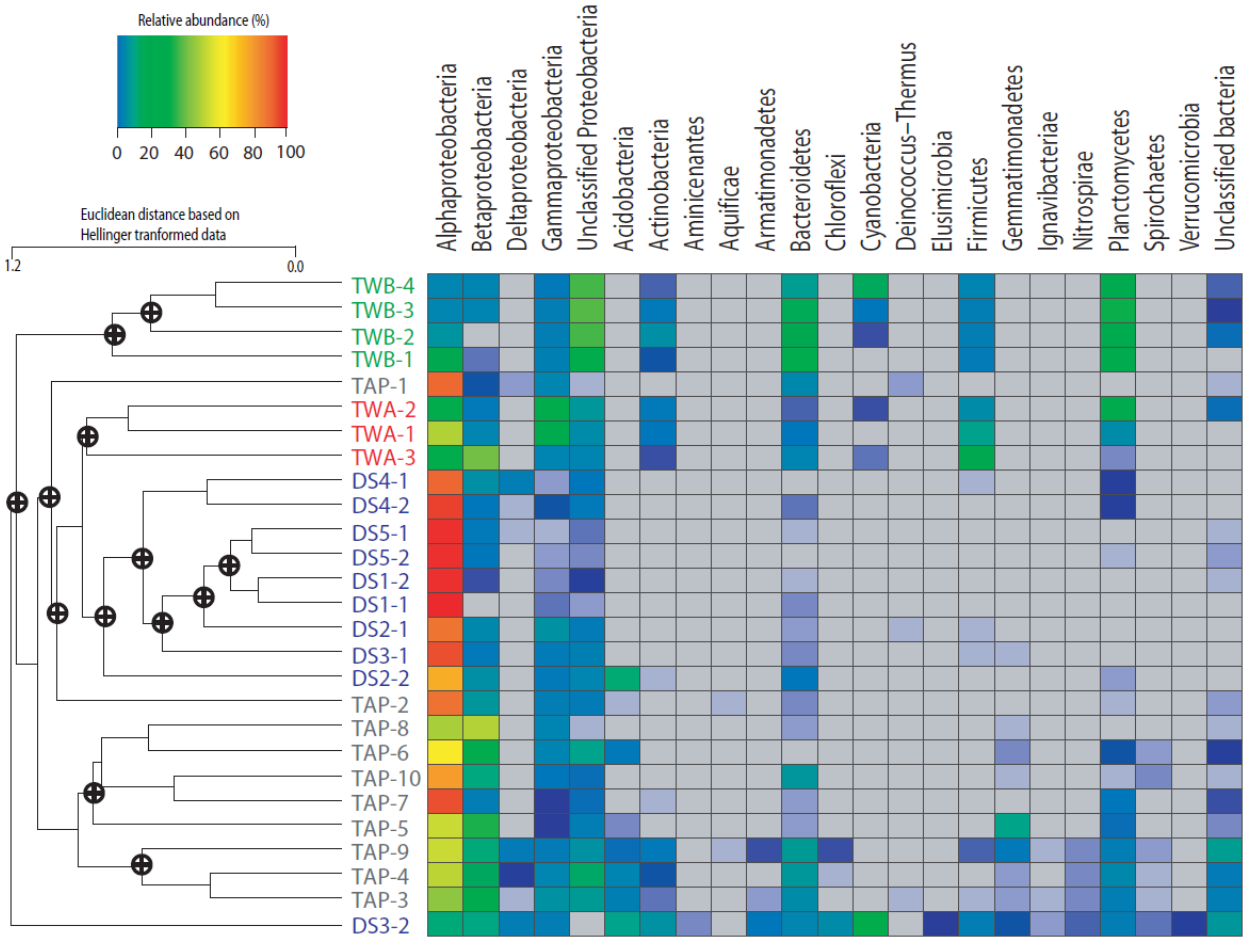

Figure 6.3: Heat map illustrates the relative abundance of different phyla and Proteobacteria classes in treated water (TW), distribution system (DS) and tap water (TAP) samples. Hierarchical clustering of samples is based on the similarity profile analysis of their bacterial community profiles (significant clusters at α=0.05). Samples with similar community structure cluster together, taking into account the relative abundance of each OTU

Six bacterial phyla were identified in TW samples (Figure 6.3). *Proteobacteria* was the dominant phylum (60±16%), based on the averages calculated for the five TW samples. The remaining phyla were *Planctomycetes* (20±12%), *Bacteroidetes* (10±9%), *Firmicutes* (6±6%), *Cyanobacteria* (2±5%), and *Actinobacteria* (2±2%). Among the classes of *Proteobacteria*, *Alphaproteobacteria* (20±17%), *Gammaproteobacteria* (10±12%), and *Betaproteobacteria* (8±14%) were identified. The classes *Planctomycetia* (20±12%) and *Sphingobacteriia* (9±9%) were also abundant in the TW samples. In addition, seven other classes accounted for 10±6% of the phyla, and unclassified *Proteobacteria* accounted for 22±16%. While the samples from both treatment plants (TWA and TWB) had the same groups of bacteria, they vary in the proportions in which they are present. *Proteobacteria* (76±8% vs. 47±3%) and *Firmicutes* (11±7% vs. 3±1%) were most abundant in TWA, whereas *Planctomycetes* (10±12% vs. 27±3%) and *Bacteroidetes* (2±2% vs. 16±6%) were most abundant in TWB. This variability likely reflects differences in treatment processes (presence of ozonation) and/or differences in chlorine dosing (TWB has a 70% higher median chlorine residual concentration than TWA). Furthermore, TWA samples exhibited greater variation in total bacterial counts (Figure 6.2C).

Eight bacterial phyla were observed in the DS sectors (excluding sample DS3-2). *Proteobacteria* phylum was the most abundant (98±4%), based on the average of the nine samples. An additional seven phyla accounted for 2±5% of the sequences. The following classes of *Proteobacteria* were identified: *Alphaproteobacteria* (92±8%), *Betaproteobacteria* (2±2%), and *Gammaproteobacteria* (1±1%). An additional nine classes accounted for 2±4% of the community. Results from our study indicate that the samples from the five different sectors analyzed were comprised of very similar microbial populations and harbored together (Figure 6.3), including DS4, which was exclusively supplied by TWB while the other sectors were supplied by a mix of TWA and TWB. DS1, DS4, and DS5 were completely dominated by *Proteobacteria* (≈100%), as were one replicate each of DS2 and DS3. The different replicate of DS2 (DS2-2) also had a higher abundance of *Proteobacteria* (87%); however, it had smaller proportions of *Acidobacteria* (12%) and *Bacteroidetes* (1%). Only DS3-2 exhibited a unique bacterial community structure in comparison to the other DS samples, which were relatively stable and homogeneous over the five sectors. DS3 presented the highest levels of total bacteria and water residence time among the DS samples. DS3-2 was composed of *Cyanobacteria* (30%), *Proteobacteria* (27%), *Acidobacteria* (10%), and *Actinobacteria* (6%).

Analyses of TAP samples enabled identification of 11 bacterial phyla. *Proteobacteria* was once again the most abundant phylum (91±9%), based on the average calculated from the ten tap water samples. *Bacteroidetes* (3±3%), *Planctomycetes* (1±1%), *Gemmatimonadetes* (1±3%), and *Acidobacteria* (1±1%) were also found in these samples. An additional nine phyla accounted for less than 1% of the sequences. *Alphaproteobacteria* (65±20%), *Betaproteobacteria* (18±15%), and *Gammaproteobacteria* (3±2%) were the most abundant classes of *Proteobacteria*. An additional 21 classes accounted for 8±11% of the sequences. The taps from premise plumbing presented very similar composition, with eight out of ten tap samples clustering very closely together (Figure 6.3). Two different taps (TAP1 and TAP2) clustered separately from the others and instead harbored with distribution system and treated water samples. These samples, which were taken from the intensive care department on the third floor of the hospital building used for our study, had the lowest total bacterial counts, and water residence times, and larger proportions of *Alphaproteobacteria* compared to the other taps. Moreover, TAP1 is located in a bathroom and TAP2 is near the entrance of the building, so these taps experience less stagnation. The bacterial structures in the other taps are similar and harbored by stagnation time and locations. TAP3 and TAP4 cluster together, and were taken from the transplantation department on the sixth floor. TAP9 was from the neonatology unit on the fourth floor. TAP3, TAP4, and TAP9 had the highest total bacterial counts and the lowest abundance of *Proteobacteria* (74%-85%) and *Alphaproteobacteria* (44%-52%). The remaining taps (TAP5, TAP6, TAP7, TAP8, and TAP10) cluster together in a significant cluster and were taken from the departments of transplant and oncology on the second and third floors, respectively. These differences between taps suggest that specific conditions at each location (hydraulics, material, patterns of use, etc.) are likely drivers in the variability of the classes of Proteobacteria present.

6.3.3 Differences between bacterial communities over time

The replicates for the treated water and distribution system sectors were collected at set time intervals. Thus, we analyzed differences between these replicates to detect any temporal variations occurring in the bacterial profiles at these locations. In general, the profiles from the treated water and distribution system replicates were stable over time. As shown in the dendrogram (Figure 6.3), replicates from each location clustered very closely. Samples of treated water presented small variabilities in the bacterial composition over time (samples were collected at three-week

intervals). All of the samples from TWA cluster together, as well as the samples from TWB. The samples from sectors in the distribution system exhibited relative stability over the three-week sampling periods. DS1, DS4, and DS5 replicates cluster together, and present very similar bacterial compositions, whereas DS2's profile varied slightly between replicates. In contrast, DS3 exhibit completely different profiles between its replicates, as well as compared to those of the other distribution system samples. The bacterial composition in the water leaving the treatment plants appeared less stable over time than the composition in the distribution system.

6.3.4 Distribution of OTUs across the sub-systems and their relationship with water quality parameters and estimators of diversity and richness

A principal component analysis (PCA) was conducted to explore potential variables that could be influencing the taxonomic profile of bacterial communities in this study (Figure 6.4). The first two axes (PC1 and PC2) of this analysis explain 53% of the variability observed in the bacterial communities. Free chlorine, total bacteria and water residence time are strongly correlated with PC2 ($p<0.05$). Free chlorine is positively correlated (0.91), while total bacteria and water residence time are negatively correlated (-0.71 and -0.81, respectively) with this axis. The distances among TWA, DS and TAP samples are most remarkable along PC1, whereas the three tested variables for all samples had no significant effect on this axis. Nevertheless, PC1 is positively correlated (0.63, $p<0.05$) with the amount of viable bacteria in TAP samples. For these samples, a correlation was also found between axis PC2 and copper ions data (-0.73, $p<0.05$). Data of viable bacteria and copper data were not available for TW and DS samples. Moreover, the community diversity and richness estimators are correlated with the levels of chlorine residual, total bacteria and water residence time when compared to the specific sub-systems ($p<0.05$). Ace estimators of TW samples are correlated with chlorine (0.86) total bacterial counts (0.82), and water residence time (0.87). Simpson indices of DS samples are correlated with chlorine (-0.63). In TAP samples, the Ace index is correlated with water residence time (0.79), and Chao and Shannon estimators are correlated with total bacterial counts (0.65 for both estimators).

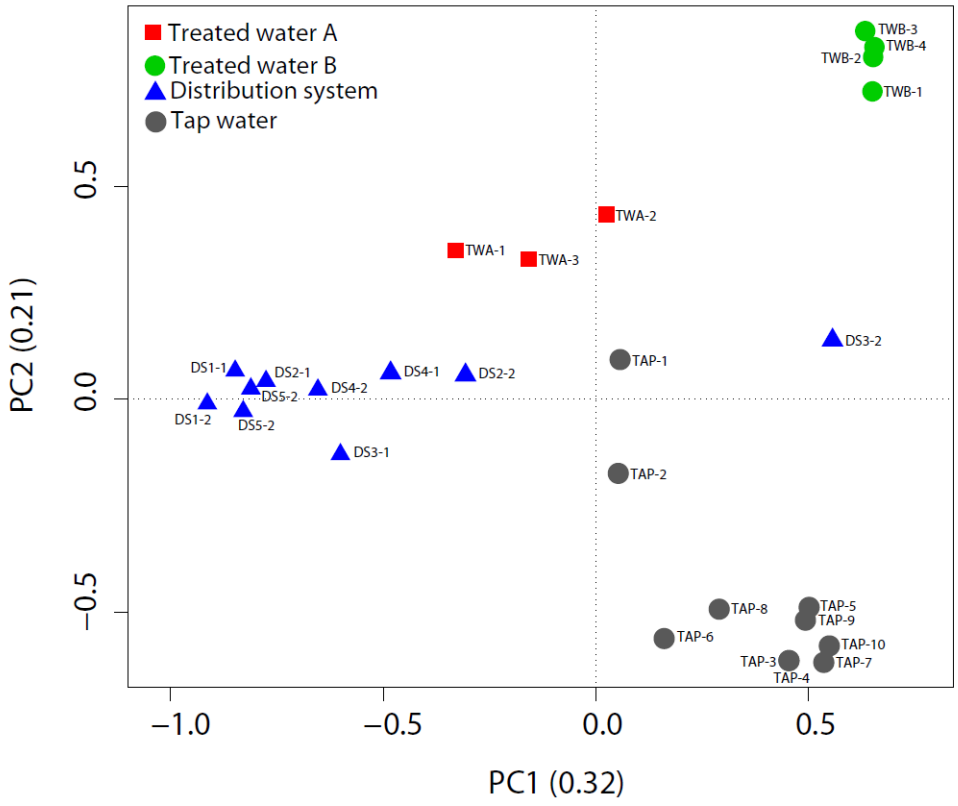

Figure 6.4: Ordination plot of principal component analysis (PCA) showing distribution of samples. Samples that cluster more closely together share a greater similarity structure. Axes PC1 and PC2 explained 53% of the variability in the data

6.3.5 Opportunist pathogens, cosmopolitan, and endemic species across the specific sub-systems

The incidence of genera known to comprise potential opportunistic pathogens (OPs) of interest was verified in the sub-systems (see Table A-5.3 in the Appendix 5). *Pseudomonas* spp. and *Sphingomonas* spp. were detected in all sub-groups (14/27 and 23/27 samples, respectively). *Mycobacterium* spp. were detected in all TW samples and one TAP sample. *Legionella* spp. were only found in tap samples at a low frequency (3/10 samples).

To verify the taxonomic richness shared among the sub-systems, and therefore identify ubiquitous species across the sub-systems, the samples were categorized as treated water, distribution system (excluding DS3-2) and taps to create a Venn diagram (see Figure A-5.1 in the Appendix 5). The

distribution system was the richest group, encompassing 39% exclusive OTUs, followed by the tap group (29%), and then the treated water group (18%). The distribution system shared 5% of OTUs with the treated water group and 5% of OTUs with the tap group. The treated water group shared 2% of OTUs with the tap group. The total shared richness between the three groups was 2%, including only members of *Alphaproteobacteria* (*Afipia* spp., *Sphingobium* spp., *Hyphomicrobium* spp., *Sphingorhabdus* spp., *Blastomonas* spp., *Methylobacterium* spp.) and *Gammaproteobacteria* (*Nevskia* spp., and *Pseudomonas* spp.).

Indicator species analysis (Indval) was employed to highlight the species (at the genus level) that best describe our three sub-systems. This type of analysis reveals the most abundant indicator in a specific sub-system that is also present in the majority of samples from that group (Dufrêne & Legendre, 1997). The outcome of this investigation can be used to identify the presence of OPs that may pose public health concern, specifically in the premise plumbing subgroup. Because of the completely different bacterial composition found in DS3-2, this sample was excluded from the indicator species analysis. The significant indicator genera with their respective number of sequences and statistically significant ($\alpha<0.05$) indicator value for each sub-system are reported in Table 6.1. In this analysis, 46 species at the genera level were associated with our three sub-systems. Thirty-eight of these species were associated with only one group (TW, DS or TAP) and eight to two groups (DS+TAP or TW+TAP). The species highlighted in this analysis for TW and TAP samples represented almost half of the relative abundance of species for these groups (44.2% and 45.3%, respectively). In contrast, for DS samples only 16.4% of its relative abundance was appointed as an indicator. This occurred because in DS samples, the most abundant family identified (*Beijerinckiaceae*), as well as other members of *Alphaproteobacteria*, could not be classified at the genera level.

Treated water presented a great variety of bacterial indicators, including 23 exclusive indicators. The most abundant genera in this sub-group were *Arcticibacter* (8.4%), *Pirellula* (4.0%), *Planctomyces* (2.7%), *Bacillus* (2.6%) and *Aquisphaera* (2.4%). The *Mycobacterium* genus (0.9%) was also identified as an indicator in this group. Only five exclusive indicators were found in the distribution system group, all but one *Proteobacteria* from the *Alphaproteobacteria* class: *Sphingorhabdus* (4.6%), *Brevundimonas* (0.7%), *Humitalea* (0.3%), and *Sphingopyxis* (0.2%). The non-*Alphaproteobacteria* indicator was from the *Deltaproteobacteria* class: *Peredibacter* (0.3%). Ten exclusive bacterial indicators were identified in the tap group, but all in relatively small

abundance. The most abundant indicators in the TAP samples were also associated with DS samples: *Sphingomonas* (1.8% in DS and 17.7% in TAP), *Afipia* (0.6% in DS and 15% in TAP), *Hyphomicrobium* (3.4% in DS and 2.7% in TAP), and *Sphingobium* (1.7% in DS and 2.7% in TAP). One indicator belonging to the *Planctomycetes* (*Gemmata*) was associated with treated water and tap groups.

6.4 Discussion

The relative influence of treated water quality on tap water quality and its hygienic implications has raised considerable attention and debate. The fundamental question raised is whether it is possible to determine the quality of water entering the distribution system that can ensure the maintenance of water quality throughout the primary and secondary distribution systems and even in premise plumbing. If premise plumbing determines the abundance and diversity of fixed and suspended biomass, then local management and on-site treatment may be the only solutions to maintain water quality up to the tap. The impact of the primary distribution system on water quality has been abundantly documented in terms of loss of disinfectant residual, bacterial indicator compliance, and bacterial biomass (Prévost, *et al.*, 2005). Industry focus was placed on biological stability, which does not take into account the hygienic implications of the bacterial communities (Prévost, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015). The new frontier is complex premise plumbing in which health relevant water quality changes can occur (Water Research Foundation (WRF), *et al.*, 2013). Clear and evident changes in the planktonic bacterial community structures are expected to occur between the water's source and water after subsequent steps of treatment (Pinto, *et al.*, 2012). Nevertheless, after treatment, results from several studies using HTS to examine changes occurring in DWDS suggest that planktonic bacterial communities leaving the plant remain relatively stable throughout distribution (Pinto, *et al.*, 2012; Lautenschlager, *et al.*, 2013; Roeselers, *et al.*, 2015), questioning the impact of water residence time and/or the presence of a disinfectant residual on microbiota. However, when it comes to premise plumbing, stagnation and pipe material have been shown to be important factors influencing the bacterial composition (Vaz-Moreira *et al.*, 2013; Ji, *et al.*, 2015).

The structure in the treated water phyla observed in this study was similar to that previously observed in the treated water from a Dutch plant (El-Chakhtoura, *et al.*, 2015), but the composition of the *Proteobacteria* classes differs. The variability observed between TWA and TWB reflects

differences in treatment processes (presence of ozonation) and/or differences in chlorine dosing (TWB has a 70% higher median chlorine residual concentration than TWA). Furthermore, TWA samples exhibited greater variation in total bacterial counts (Figure 6.1C). Predominance of *Proteobacteria* has been observed in other chlorinated and unchlorinated DWDS (Hwang, *et al.*, 2012; McCoy & VanBriesen, 2012; Pinto, *et al.*, 2012; Douterelo, *et al.*, 2013; Lautenschlager, *et al.*, 2013; Liu, *et al.*, 2013; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015). However, the predominant classes of *Proteobacteria* vary from one network to another. *Alphaproteobacteria* are usually predominant in chlorinated systems (McCoy & VanBriesen, 2012; Lu, *et al.*, 2013; Douterelo *et al.*, 2014). *Cyanobacteria* are also found in chlorinated DWDS samples, indicating that they can survive passage through different physical and chemical treatments and enter networks (Hwang, *et al.*, 2012; Lu, *et al.*, 2013). The community shifts observed in our investigated network could be explained by hydraulic disturbances from loose deposits and sediments. Changes in hydraulic conditions can affect bacterial structures and composition influencing the profiles of both water and biofilm communities (Douterelo, *et al.*, 2013). Among the ribotyping profiles we examined, DS3-2 was an outlier composed of *Verrucomicrobia* (0.7%), *Aminicenantes* (0.3%), and *Elusimicrobia* (0.8%). A small proportion of *Verrucomicrobia* (4%) has previously been reported in chlorinated drinking water (Lu, *et al.*, 2013). *Elusimicrobia* and *Verrucomicrobia* have been reported in an unchlorinated DWDS (Lautenschlager, *et al.*, 2013).

Table 6.1: Number of sequences of indicator species (at genus level) in treated water, distribution system and taps (α=0.05)

Phylum	Class	Order	Family	Genus	Indval	TW	DS	TAP
Acidobacteria	*Acidobacteria_Gp4*	*Gp4*	*Gp4*	*Gp4*	0.71	0	0	73
Actinobacteria	*Actinobacteria*	*Actinomycetales*	*Mycobacteriaceae*	*Mycobacterium*	1.00	64	0	1
			Propionibacteriaceae	*Propionibacterium*	0.62	6	1	0
Bacteroidetes	*Bacteroidetes_incertae_sedis*	*Ohtaekwangia*	*Ohtaekwangia*	*Ohtaekwangia*	0.71	0	0	16
	Sphingobacteriia	*Sphingobacteriales*	*Sphingobacteriaceae*	*Arcticibacter*	0.75	597	7	0
				Pedobacter	0.66	39	0	0
			Chitinophagaceae	*Flavisolibacter*	0.71	0	0	42
				Sediminibacterium	0.78	0	0	27
Cyanobacteria	*Cyanobacteria*	*Family II*	*Family II*	*GpIIa*	0.66	145	0	0
Firmicutes	*Bacilli*	*Bacillales*	*Bacillaceae 1*	*Bacillus*	0.93	181	0	0
	Clostridia	*Clostridiales*	*Clostridiaceae 1*	*Anaerobacter*	0.76	107	0	0
			Peptostreptococcaceae	*Clostridium XI*	0.85	26	0	0
Gemmatimonadetes	*Gemmatimonadetes*	*Gemmatimonadales*	*Gemmatimonadaceae*	*Gemmatimonas*	0.83	0	1	127
Planctomycetes	*Planctomycetia*	*Planctomycetales*	*Planctomycetaceae*	*Aquisphaera*	0.93	173	0	0
				Blastopirellula	0.66	13	0	0
				Gemmata	0.87	176	2	48
				Isosphaera	0.66	10	0	0
				Pirellula	0.92	287	0	9
				Planctomyces	0.90	192	12	0
				Rhodopirellula	0.85	20	0	0
				Singulisphaera	0.66	10	0	0
				Telmatocola	0.84	48	0	1
				Zavarzinella	0.91	65	0	3
Proteobacteria	*Alphaproteobacteria*	*Caulobacterales*	*Caulobacteraceae*	*Brevundimonas*	0.67	0	62	0
		Rhizobiales	*Beijerinckiaceae*	*Beijerinckia*	0.76	0	24	54
			Bradyrhizobiaceae	*Afipia*	0.99	18	56	1523
				Bosea	0.91	23	144	69
			Hyphomicrobiaceae	*Hyphomicrobium*	0.97	23	311	274
			Phyllobacteriaceae	*Mesorhizobium*	0.76	24	0	0
		Rhodospirillales	*Acetobacteraceae*	*Humitalea*	0.58	0	25	0
			Rhodospirillaceae	*Dongia*	0.77	60	16	0
		Rickettsiales	*Rickettsiaceae*	*Orientia*	1.00	171	0	0
		Sphingomonadales	*Erythrobacteraceae*	*Altererythrobacter*	0.88	0	3	76
			Sphingomonadaceae	*Novosphingobium*	0.77	0	10	61
				Sphingobium	0.94	38	159	271
				Sphingomonas	0.99	22	164	1788
				Sphingopyxis	0.58	0	16	0
				Sphingorhabdus	0.94	41	415	1
	Betaproteobacteria	*Burkholderiales*	*Burkholderiaceae*	*Chitinimonas*	0.66	465	0	0
			Burkholderiales_incertae_sedis	*Aquabacterium*	0.69	1	0	36
			Comamonadaceae	*Delftia*	0.60	8	2	0
		Ferrovales	*Ferrovaceae*	*Ferrovum*	0.71	0	0	11
	Deltaproteobacteria	*Bdellovibrionales*	*Bacteriovoracaceae*	*Peredibacter*	0.58	0	29	0
	Gammaproteobacteria	*Candidatus Carsonella*	*Candidatus Carsonella*	*Candidatus Carsonella*	1.00	74	0	0
		Xanthomonadales	*Sinobacteraceae*	*Nevskia*	0.88	7	33	71
Spirochaetes	*Spirochaetia*	*Spirochaetales*	*Leptospiraceae*	*Leptospira*	0.63	0	0	7

Indicators in treated water
Indicators in distribution system
Indicators in premise plumbing
Indicators in distribution system and premise plumbing
Indicators in treated water and premise plumbing

Our results show that the specific environmental conditions (levels of chlorine, bacterial counts, pipe material, water residence time, and biofilm/water interface) of each sub-system are determinants of the bacterial community composition, promoting or inhibiting the growth of specific organisms. In the water's passage from treatment plants to taps, *Proteobacteria* increased 40% in the distribution systems, and decreased 7% in the premise plumbing. Despite the superior proportion of *Proteobacteria* in both distribution system sites and taps, the distribution profile of OTUs encompassing this phylum differed between both locations. While DS samples were clearly dominated by *Alphaproteobacteria* (92%), in premise plumbing the loss of disinfectant residual and premise plumbing conditions resulted in a decrease in *Alphaproteobacteria* (65%) and allowed the growth of *Betaproteobacteria* (18% vs. 2% in DS) and other classes that were less abundant or absent in the distribution system. Similar responses of *Alphaproteobacteria* and *Betaproteobacteria* to disinfectant residuals have been previously reported (McCoy & VanBriesen, 2012). Members of *Alphaproteobacteria* seem to exhibit a higher resistance to chlorine and are found in abundance in chlorinated networks. In contrast, the opposite is observed for *Betaproteobacteria*. The latter group grows more readily in low disinfectant conditions and has been found to be more abundant in unchlorinated distribution systems (Liu, *et al.*, 2013; El-Chakhtoura, *et al.*, 2015) and in biofilms in chlorinated systems (Douterelo, *et al.*, 2013).

Other studies examining temporal variations in bacterial communities have also observed stable profiles over short and long periods in unchlorinated DWDS (Lautenschlager, *et al.*, 2013; El-Chakhtoura, *et al.*, 2015). However, a previous study investigating variations in a chlorinated distribution system verified temporal variability in spring and summer samples, primarily due to changes in disinfectant levels in response to temperature fluctuations (McCoy & VanBriesen, 2012). The percentages of shared richness found in this study were much lower than those found in an unchlorinated system (El-Chakhtoura, *et al.*, 2015), where 58% to 65% of OTUs were shared between treated water and the water from one location in the distribution system. This difference is probably explained by the higher variation in water quality, as influenced by the presence/absence of chlorine in the treated water at the plants, in the distribution system and at the taps.

Several of the identified indicators in our study have been reported in previous studies. Representatives of the genus *Mycobacterium* include opportunist pathogens that are generally highly resistant to disinfectants (Vaerewijck *et al.*, 2005). They have been detected as the most

abundant genus in 89% of flushed tap samples from 16 cities in the United States using chlorine and monochloramine as disinfectants (Holinger, *et al.*, 2014). The presence of *Bosea*, *Hyphomicrobium*, *Sphingomonas*, *Nevskia*, *Mycobacterium*, *Mesorhizobium*, *Aquabacterium*, and *Sphingopyxis* has been reported in bulk water and biofilm samples in a simulated distribution system using chlorine as a disinfectant (Douterelo, *et al.*, 2013). *Nevskia* was found to be more abundant in highly varied flow conditions (Douterelo, *et al.*, 2013) and in samples from a pipe break event (McCoy & VanBriesen, 2012). This could explain its association with distributed water and premise plumbing groups, where water usage patterns are highly variable between night and day, and between weekdays and weekends.

Sphingomonas and *Afipia* are both reported to have significant impact in healthcare settings (La Scola *et al.*, 2000; Lin *et al.*, 2010; Ryan & Adley, 2010). A few species of *Sphingomonas* are considered to have clinical significance, with *Sphingomonas paucimobilis* being the most important. These bacteria can survive in low nutrient environments and have the ability to form biofilms in water piping. This pathogen can cause various types of infections, often bacteremia/septicemia (Ryan & Adley, 2010). *Sphingomonas* spp. are resistant to disinfection, produce extracellular polymeric substances, and can rapidly colonize pipe surfaces (Zhang *et al.*, 2012). A predominance of *Sphingomonas* spp. and *Sphingobium* spp. with antibiotic resistance have been previously detected in water samples from taps (household and hospital) and dental chairs in a university dental school clinic (Vaz-Moreira *et al.*, 2011). In addition, their tolerance to high levels of copper ions enables them to not only survive in copper piping, but also fully thrive, as well as, promote copper corrosion (Pavissich *et al.*, 2010). In an unchlorinated system, *Sphingomonas* spp. were predominant in the pipe wall biofilm, in loose deposits and suspended solids. *Sphingomonas* spp. are also the second most important genera detected in bulk water (Liu, *et al.*, 2014). The predominance of *Sphingomonas* spp. in first flush tap samples from a large building could be attributed to the contribution of the biofilm in this section of the premise plumbing, where smaller pipe diameters increase the proportion of biofilm released after short stagnation times, as is often encountered in hospital environments.

The second most important indicator species in taps were *Afipia* spp., a water-associated amoeba-resisting bacteria of public health significance. *Afipia* spp. have been isolated from a hospital water supply (La Scola & Raoult, 1999; La Scola, *et al.*, 2000) through co-culture with amoeba. *Afipia* spp. are suspected to be involved in pneumonia since several patients with acute pneumonia had

elevated levels of anti-*Afipia* antibodies (Bousbia *et al.*, 2013). *Gemmata massiliana*, found as an indicator in TW and TAP sub-systems, has been isolated from two hospital water networks in France (Aghnatios & Drancourt, 2015). The frequent detection of this pathogen in two hospital networks suggested the potential exposure of patients and health-care workers to this organism. Thus, the authors of that study suggested evaluating this organism as a potential opportunistic pathogen of public health significance.

The results from genera countaining OPs and indicator analyses remain difficult to interpret, since these sequences may not be representative of organisms that cause relevant health effects. However, this can be investigated further through follow-up environmental sampling, helping clinicians identify sources of nosocomial pneumonia.

Overall, the comparative analysis of the diversity and relative abundance of the planktonic bacterial community in a full-scale DWDS using an HTS approach reveals three main findings. First, we have shown that the bacterial community profile in treated water, distribution system sectors, and taps in premise plumbing are diverse and clearly distinct at different taxonomic levels. Second, we have linked noticeable variations in microbial communities to changes in the concentration of disinfectant residual, total bacterial counts, and water residence times. Finally, the use of indicators revealed the presence of important markers in different groups of DWDS samples. Although these findings provide relevant information, they are contingent upon the sensitivity of the methods used. More importantly, our results only inform on the diversity of suspended bacteria, not of the biofilm.

The combination of methods used in this study have a high potential for drinking water quality investigative monitoring to further our understanding of distribution system ecology. The generated information is needed to understand where utilities can act to prevent the growth of OPPPs. Distribution conditions that may be linked with the prevalence of health relevant specific bacterial groups or species could be identified using the methods described in this study. Additional research is needed to understand the drivers of the shifts of microbial communities in suspended and fixed microbiomes within a full-scale DWDS and premise plumbing.

6.5 Acknowledgements

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CHAPTER 7 GENERAL DISCUSSION

This chapter presents a critical discussion of the main findings of this doctoral thesis. The overall objective was to measure the impact of DMAs implementation on water quality in five temporary DMAs in a full-scale DWDS and to develop a strategy to limit sampling campaigns in the DMA area. Figure 7-1 summarizes the different steps of the research completed and their relevance to the specific objectives pursued. The first step was to quantify the impact of DMAs implementation on hydraulic conditions and multi-water quality parameters. Based on these results, obtained from extensive and detailed monitoring in different types of locations, it was possible to identify the parameters and locations most affected by DMAs implementation. Once these aspects were established, the second step was to guide future monitoring in DMAs combining limited field sampling, hydraulic modeling, and water quality modeling to predict changes in regulatory compliance for disinfection by-products (DBPs). Based on the results obtained from the bacterial communities in the DMAs, the last step was to understand whether treated water was determinant in the communities detected along the system, as well as the influence of stagnation conditions in premise plumbing. Conclusions and recommendations for DMAs implementation concerning water quality will be addressed in Chapter 8.

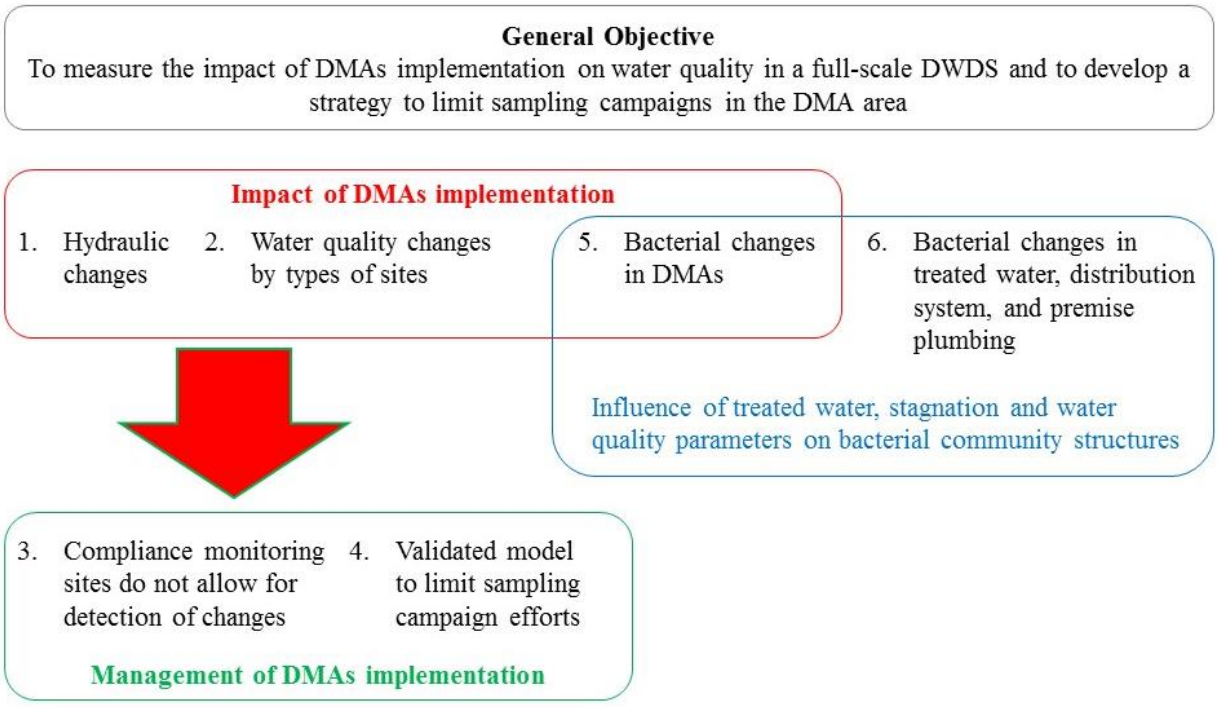

Figure 7.1: Summary of the research conducted

7.1 Impact of DMAs implementation

As discussed in Chapter 2, the potential changes associated with the closing of valves to the establishment of DMAs include hydraulic conditions and water quality inside the DMA and near its boundaries. Scarce available literature suggests that there is no significant impact with overall no change or better water quality after implementations. This thesis presents additional data summarized in this section to address the first five research questions (Chapter 3):

- Do hydraulic conditions change significantly after DMAs implementation?
- Which locations are more affected by DMAs implementation?
- Does monitoring at compliance monitoring sites allow for the detection of water quality changes?
- Which water quality parameters are influenced the most by the physical and hydraulic changes?
- Are changes in bacterial communities after DMAs implementation in new stagnant sites comparable to existing ones?

7.1.1 Impact of DMA implementation on hydraulic conditions

In this project, the impact of DMAs on hydraulic conditions such as velocities, WRT, and flow direction, that subsequently can impact water quality, was accessed by simulations in WaterGEMS using calibrated models for each DMA. These changes are expected following the closing of valves to form the DMA boundaries. Limiting the sites with high WRT and/or stagnation is a priority since the maintenance of water quality in these locations is a challenge for water utilities. During the design of the DMAs studied in this project, efforts were made by the water utility personnel to control the number of closed valves and try to limit the boundaries close to major consumers. Due to these efforts, minimal to moderate variations were observed in overall hydraulic conditions (Chapter 5).

In four out of five DMAs the changes in water residence times were limited within a two-hour range (68 to 99% of nodes), with combined increases and decreases of water age at minimum and maximum demand conditions. Only in DMA 3, more important WRT variations (up to 10 hours) were observed at 55% of nodes. The number of nodes with very high WRT (> 50 hours) increased

by approximately 40% in some DMAs (2 and 3) and by about 10% in DMAs 4 and 5. In DMA 1 this proportion did not change. This was expected since DMA 1 was almost already isolated and only three valves were closed, while between 14 and 31 valves were closed in the other DMAs to form the boundaries. No change on water velocities was observed between 18 and 48% of the length of pipes after closing the valves in all DMAs in the maximum demand conditions. Minimal changes (about 0.05 m/s) were predominant in all DMAs. In DMA 3, increases in water velocity greater than 0.15 m/s were observed on more than 20% of the network length, in the maximum demand conditions, mainly due to modifications of the supply pipes in this sector. Changes in flow direction were observed mainly in DMAs 3, 4, and 5 (8, 16 and 10% of the length of pipes, respectively), while in DMAs 1 and 2 changes were less than 3% of the length of pipes.

The overall impact of hydraulic changes in these pilot DMAs ranged from negligible to moderate. These results partially invalidate hypothesis #1 that the implementation of DMAs does not change the overall distribution of hydraulic parameters. However, it must be noted that these changes are modest. Moreover, the results obtained in this project are limited to the configuration of these DMAs without active pressure management. Pressure management was not installed at the time of the pilot DMA program, as pilot implementation aims to confirm the feasibility of the proposed DMA configuration. Since flow is dependent on the pressure in the system, when pressure management is applied slower water velocities are expected and consequently higher water residence times and increased risk of stagnation are likely to occur.

Our results are in agreement with previous reports that overall hydraulic conditions do not vary greatly. Other studies investigating hydraulic changes produced by DMAs verified lower velocities near to closed valves (UK Water Industry Research (UKWIR), 2000) and important variations in WRT by node (Grayman, *et al.*, 2009). Nevertheless, they reported insignificant hydraulic variations when considered overall results.

7.1.2 Effects of DMA implementation on water quality

Until now, studies investigating the impact on water quality from the implementation of DMAs in DWDS established their conclusions based on scarce evidence from limited monitoring campaigns (UK Water Industry Research (UKWIR), 2000; Fanner, *et al.*, 2007; Kunkel & Sturm, 2011). For our investigation, an enhanced water monitoring plan was drawn and deployed as a full-scale field study during the temporary implementation of five pilot DMAs. This investigation was carried out

taking into account the hydraulic characteristics of diferent sampling points. Multiple water quality parameters as well as bacterial abundance and community structures were investigated before and after DMAs implementation (as described in Chapters 3, 4 and 5).

The choice of representative sampling sites is a critical element of a monitoring plan capable of investigating variations in water quality, since the location chosen could mask or influence the perception of the DMA's impact. In this study, various types of sampling sites were chosen to cover the potential changes caused by the DMA implementation based on hydraulic simulation results, the characteristics of each DMA, and using the experience of the water utility staff. In our detailed sampling studies, sampling locations were assigned to one of the seven groups: inlets, existing and new created dead-ends after the closing of valves, outside DMA (near the boundaries), extremities, sites with changes in flow direction or water age variation, and compliance monitoring sampling points utilized by the water utility.

Results of water quality monitoring at the inlet points of each DMA establish the reference values. These baseline values were important to determine if observed changes in water quality from other sites can be attributed to the closing of valves or if they are caused by changes upstream the DMAs area. In this study, due to the strategy of chosen sites representatives of inlets, it was possible to fully take into account the upstream water quality changes occurring in the system during the course of the monitored period. These upstream changes resulted in water quality changes in the DMA sector that were not caused by the closure of valves (Chapters 4 and 5). Monitoring at inlet sites demonstrated that various parameters were influenced by seasonal patterns, variations at treatment plants and/or another water quality issue caused by the distribution system itself upstream the area (validating hypothesis #2). The monitoring at dead-ends showed that the existing dead-ends tend to remain stable while some of the new dead-ends sometimes reached the same or even worse degradation, also because of elevated WRT. Extremities and sites outside the DMA delineation but close to the boundaries were also impacted by the implementations. We did not observe any significant impact at sites selected to investigate changes in flow direction. However, it is possible that transient water quality changes occurred at these sites immediately following the closing of the area. We observed that, by the time the first monitoring after the implementation was done (normally one week after the closing of valves), flow and water quality conditions were already stable. These short-term water quality variations would only be detectable with online monitoring. Finally, this monitoring strategy proved that compliance monitoring sites are not suited

to detect water quality changes following DMAs implementation, validating hypothesis #6. These sites are normally located at the extremities of the distribution system. However, as they were located inside public buildings, it is possible that water quality was also influenced by the premise plumbing conditions.

Chapter 4 shows that the main effects of DMAs implementation on water quality were observed for disinfectant residuals, turbidity, iron, and manganese at specific types of locations (validating hypothesis #3). As expected, lower concentrations of disinfectant residuals were clearly more frequent at points where WRTs increased after the closing of valves. Strong correlations between these variables were observed. Higher concentrations of metals and peaks of turbidity are influenced by hydraulic conditions. Therefore, the timing of field sampling is important. In our case, samples were collected at maximum demand conditions. Higher demand could influence the transport of particles (previously settled during minimum demand conditions) causing them to be released in the bulk water at these locations. This accumulation should also be considered in terms of potential impact on microbial quality. The presence of organic and inorganic particles can protect bacteria from disinfectant residuals. These particles can be suspended with variation in demand or transients in the system and reach consumer's tap. Higher iron and manganese concentrations in bulk water can result in discoloration (Slaats, et al., 2003; Vreeburg & Boxall, 2007) and will be perceived negatively by customers. Studies investigating historical data of DMAs concluded that they could influence discoloration (Armand, *et al.*, 2015) as well as cause significantly higher customer complaints during summer when water consumption and water temperatures increase (Prasad & Danso-Amoako, 2014). Even if the concentrations of iron increased, they only exceeded the recommended levels for iron (300 µg/L) (Health Canada, 2014) five times in two DMAs. Concentrations of manganese remained below recommended aesthetic recommendations (50 µg/L). Concentrations of these metals in drinking water are not regulated in Canada because they are considered to have little or no adverse health impact. In the future, manganese levels could be regulated on a health basis. Indeed, epidemiological studies have strongly suggested significant neurological effect in children (Wasserman, *et al.*, 2006; Oulhote, *et al.*, 2014), not unlike those of lead, could result from exposure in drinking water.

In Chapter 5, the effects of DMAs implementation were assessed in terms of bacterial abundance and community structures. The impact of higher WRT or stagnation on bacterial abundance in new dead-ends were observed in some DMAs while water quality and bacterial abundance mostly

remained constant at existing dead-ends. The analysis of bacterial community structures showed that the taxonomic variations were influenced by the specific hydraulic and physicochemical conditions of each DMA. Detailed monitoring of sites entering the sector revealed significant water quality variations that were attributed mainly to water quality changes upstream the DMAs. Parameters that were clearly influenced by changes in treatment plants, season variations or by the distribution system itself were temperature, pH, DOC, free chlorine residuals, THM4, and total bacteria. These results were observed at maximum demand conditions (time chosen for sampling). The release of deposition (turbidity, metals) was observed during our monitoring in locations where low flow conditions and higher WRTs were expected showing the influence of demand. Thus, since turbidity and metals were correlated with bacterial loads, increased bacteria levels were expected. These trends were much clearer for HPCs, which reflects the impact of chlorine on culturability. As the timing of sampling could bias or mask the perception of DMAs' implementation impact, additional investigation on the influence of demand conditions is needed.

A predictive model was constructed to guide future monitoring in DMAs combining optimized field sampling, hydraulic modeling, and water quality to predict changes in regulatory compliance for disinfection by-products (DBPs). The predictive model was constructed using multiple linear regression with the data collected from the first four DMAs (DMAs 1-4). The best R^2 (0.91) was found using non transformed data and the significant variables were free chlorine, THM4 concentrations at upstream sites and the variation of water residence time between the sites (Chapter 4). Subsequently, the validation of the model was done using data (free chlorine, THM4, and WRT values) from one inlet site at DMA 5 and predicting THM4 values for the six sampling dates at all nine other sites using only the water residence time from these sites. A strong correlation (0.60) was obtained using the predictive model from DMAs 1-4 to obtain THM4 values in DMA 5, validating hypothesis #7. This model is valid for a wide range of conditions: free chlorine concentrations ranging between 0.25 mgCl_2/L and 1.12 mgCl_2/L, THM4 values ranging from 11.7 μg/L to 83.5 μg/L at *i* sites; predicted THM4 19.3 μg/L to 89.9 μg/L, and ΔWRT_{ij} from 0.10 to 41.4 h. This model can be used by the water utility to improve their understanding on the water quality impact caused by DMAs implementation and optimize the monitoring in these areas.

7.2 Bacterial communities across DWDS

Observed differences of bacterial communities across DWDS were interpreted to answer two research questions (Chapter 3):

- Does treated water determine bacterial communities in distribution systems and premise plumbing from a large building?
- Which water quality parameters influence bacterial communities the most?

We evaluated whether bacterial communities detected in treated water determine community structures in main pipes and premise plumbing via HTS of PCR-amplified 16S rRNA genes. The impact of the primary distribution system on water quality has been abundantly documented in terms of loss of disinfectant residual, bacterial indicator compliance, and bacterial biomass (Prévost, *et al.*, 2005). Industry focus was placed on biological stability, which does not take into account the hygienic implications the bacterial communities select (Prévost, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015). The new frontier is complex premise plumbing in which health relevant water quality changes can occur (Water Research Foundation (WRF), *et al.*, 2013). Clear and evident changes in the planktonic bacterial community structures are expected to occur between the water's source and water after subsequent steps of treatment (Pinto, *et al.*, 2012). Nevertheless, after treatment, previous studies using HTS showed a consistency in planktonic bacterial composition from treated water to locations in DWDS (Pinto, *et al.*, 2012; Liu, *et al.*, 2014; Prest, *et al.*, 2014; El-Chakhtoura, *et al.*, 2015; Roeselers, *et al.*, 2015), even in networks using residual disinfectants. Some variations were observed only at locations with higher water residence time (Lautenschlager, *et al.*, 2013). These results question the impact of water residence time and/or the presence of a disinfectant residual on microbiota. However, when it comes to premise plumbing, stagnation and pipe material have been shown to be important factors influencing the bacterial composition (Ji, *et al.*, 2015).

Results from Chapter 6 show significant differences on the planktonic bacterial communities present in the treated plant effluent, water in main pipes, and water from premise plumbing, validating hypothesis #5. Our results support that the specific environmental conditions (levels of chlorine, bacterial counts, pipe material, water residence time, and biofilm/water interface) of each sub-system determine bacterial community composition, promoting or inhibiting the growth of

specific organisms. Moreover, as demonstrated in Chapter 5 where a more comprehensive investigation was made on the influence of environmental factors affecting bacterial community structures, other influent parameters such as manganese, iron, HPC, DOC, and turbidity were identified.

7.3 Management of DMAs implementation

Since there are numerous locations that can be selected for sampling during a DMA investigation, the choice of sampling points is critical to yield results that are representative of the changes occurred in the area. As previously discussed, compliance monitoring sites are not suited to detect changes caused by DMAs implementation.

In order to optimize cost and time, we proposed an investigative approach that provides key answers to distribution system managers while limiting the cost of extensive additional water quality monitoring after the implementation of a DMA.

The work presented in this thesis provides key elements of an investigative approach to assist utilities in predicting and managing water quality after DMA implementation:

- Hydraulic modeling to easily predict areas where water quality changes are likely to occur and provide an estimate of the importance of increase of the number of water quality critical points (locations with elevated WRT),
- Targeted sampling to evaluate water quality issues in critical points of the distribution system can provide an assessment of anticipated water quality issues after the implementation of DMAs. This is especially important in terms of predicting the likelihood of increased levels of HPCs at elevated WRTs,
- The use of a site specific chlorine consumption/DBP model to predict the potential for DBPs exceedances after DMA implementation.

We believe that such approach can be easily implemented by utilities and can provide a site-specific tool to anticipate the impact of DMA implementation on water quality.

7.4 Study limitations

The results discussed in this thesis come from essays carried out in the field where several factors beyond the scope of our study occur and influence them. Thus, this thesis presents some limitations concerning DMAs implementation and bacterial communities:

- Hydraulic models were calibrated using pressure and flow tests in fire hydrants from the field for adequate flow in the system (fire-fighting requirements). No additional test was done to confirm WRTs. Moreover, since some sampled dead-ends did not present water demands in the models, water demands were assigned to these sites in order to avoid linear increase in the WRT;
- Pressure management was not applied during DMAs implementation;
- Water quality was only measured at maximum demand conditions;
- The effect of DMAs implementation was verified after one week of the closing of valves and the maximum study period was 16 weeks. Thus, short-term and long term impacts of closing valves were not studied;
- Bacterial communities in bulk water were evaluated only once before and once after implementations. Moreover, the samples were pooled together by DMA or by three types of sites (inlets, existing and new dead-ends);
- Our investigations and resulting conclusions are limited to the planktonic bacterial communities. Although they offer some insight into the dynamics of bacterial communities, they cannot be used to conclude on trends in the biofilm, the dominant microbial phase in the distribution system. Additional research is needed to understand the drivers of the shifts of microbial communities in suspended and fixed microbiomes within a full-scale DWDS and premise plumbing.

CHAPTER 8 CONCLUSIONS AND RECOMMENDATIONS

This thesis sought to better understand the impact of DMAs implementation on water quality in DWDS and to guide future monitoring of DMAs area. Several questions were initially raised: do hydraulic conditions change significantly after DMAs implementation? Which water quality parameters are influenced the most by the physical and hydraulic changes? Which locations are more affected by DMAs implementation? Does monitoring at compliance monitoring sites allow for the detection of water quality changes? Are changes in bacterial communities after DMAs implementation in new stagnant sites comparable to existing ones? Does treated water determine bacterial communities in distribution systems and premise plumbing from a large building? Which water quality parameters influence bacterial communities the most? Through extensive and detailed field monitoring supported by hydraulic simulations, the results from this research brought evidences which helped to answer these questions.

The following conclusions were reached regarding the impact of DMAs implementation in hydraulic conditions:

- Changes in WRT varied with the number of closed valves and DMA configuration but minor changes were predominant in these monitored DMAs;
- Hydraulic simulations showed significant increase (10-40%) of locations with high WRT (>50 hours) in 4/5 pilot DMAs;
- Water velocities presented minor variations;
- Overall, the hydraulic changes caused by the isolation during the temporary implementation of DMAs were minimal to moderate and their extent depended on the configuration of the DMA.

The following findings were established from detailed and extensive monitoring on the water quality impact of DMAs implementation:

- Disinfectant residuals, turbidity, iron and manganese were the most affected monitored water quality parameters after DMAs implementation;
- Noticeable changes were detected in locations with high WRT, or near the boundaries such as created dead-ends, outside sites, and extremities;

- Some created dead-ends and outside sites showed water quality similar or worsen than the monitored existing dead-ends;
- Slight increase in THM4 concentrations were observed after the implementations, but the main changes in this parameter were attributed to water quality variations from treatment plants;
- Bacterial abundance investigations clearly showed that dead-ends were critical sites for HPCs, and in a minor level for total cell counts;
- Factors affecting culturability (chlorine residual, DOC, metals, etc.) were determinant in the changes of the taxonomic compositions;
- Bacterial abundance and community structures in bulk water reflected local water quality conditions;
- Temperature, pH, DOC, chlorine residual, DBPs, and total bacteria were clearly influenced by seasonal patterns, variations at treated water, and/or by the distribution system itself before DMAs area;
- The implementation of DMAs was associated with an apparent susceptibility to cause discoloration events;
- Overall, no important water quality impact was noticed.

The following conclusions were founded about bacterial community structures from treated water to tap:

- Planktonic bacterial community structures in treated water, distribution system, and premise plumbing were diverse and distinct at different taxonomic levels;
- Specific environmental conditions (disinfectant residual, bacterial abundance, pipe material, water residence time, and biofilm/water interface) of each sub-system were important factors that promote or inhibit the development of specific microorganisms of hygienic relevance.

The following recommendations are proposed concerning the modeling, design, and future monitoring of DMAs:

- A calibrated model is indispensable when designing DMAs since locations such as dead-ends are the most critical sites because of poor demand estimations;
- A realistic demand should be assigned to dead-ends in order to avoid linear increase in the WRT when running hydraulic simulations. Field water quality data clearly showed how inadequate these estimations were;
- The number of stagnant sites should be minimized as much as possible when designing DMAs or the valves should be located near larger consumers;
- The incidence of possible episodes of water quality deterioration when implementing DMAs can be quantified by combining hydraulic modeling and documenting the quality at existing high risk sites by targeted monitoring;
- Changes in water quality entering the sector should be accounted for when investigating water quality in these areas;
- Sampling sites must be carefully chosen to avoid a false perception of DMAs impact. As observed by our monitoring, compliance monitoring sites even if located at extremities are not suitable to detect potential changes caused by DMAs implementation.
- The impact on water quality and hydraulics outside of the isolated areas should also be considered;
- The simple approach proposed in this study to predict THM4 in a DMA area using only inlet data could be a practical tool for the water utility avoiding continuous and excessive monitoring;
- Online monitoring can be very practical to detect changes associated to consumption.

This thesis generated new questions and ideas for future research. It would be interesting to:

- Evaluate the additional effect on hydraulic conditions and water quality of pressure management on DMAs;
- Understand the influence of demand conditions in water quality before and after the implementation of DMAs;

- Investigate the interactions between bacterial communities from bulk water and biofilm and its sanitary significance in DMAs sites with variable hydraulic conditions;
- Evaluate the water saved from DMAs implementation associated with pressure management versus the water employed for flushing in impacted locations.

The increased water needs and the decrease of available resources forces water utility managers to optimize their systems with cost-effective techniques. Nevertheless, since drinking water is an important concern for public health, it is essential to comprehend the impact caused by the employment of these practices. Continuous research efforts and the use of innovative procedures will help further advance our understanding of complex interactions in DWDS, where numerous variables influence the water quality.

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APPENDICES

APPENDIX 1 – SUPPLEMENTAL INFORMATION: CALIBRATION OF HYDRAULIC MODELS

Title: Procédure de calibration des modèles hydrauliques utilisée par la ville de Montréal

Author: Idriss Lahnin, ing. PMP – Service de l'eau – Division de l'optimisation du réseau – Ville de Montréal

Number of pages: 3

Procédure de calibration des modèles hydrauliques utilisée par la ville de Montréal

L'utilité principale des modèles est la simulation hydraulique pour les calculs des pressions statiques et de la capacité hydraulique du réseau pour la défense incendie, ainsi la calibration est basée sur des essais débits/pressions aux bornes d'incendie.

La procédure de calibration des modèles hydrauliques a impliqué les étapes et considérations suivantes :

1. La mesure de la distribution du secteur : secteur isolé avec un certain nombre d'entrées et/ou sorties (sur raccords au primaire et conduites secondaires) puis mesure des débits et pressions par des débitmètres à insertion et longueurs de pressions ;
2. Établissement du bilan de consommation pour évaluer la distribution moyenne journalière (DMJ), les courbes de modulation horaire de la demande, les têtes d'eau supplémentaires, ainsi que leurs courbes de variation sur 24h lesquelles sont converties en niveau d'eau (HGL) ;
3. Les courbes de modulation horaire de la demande sont affectées aux nœuds avec consommation diffuse (consommation résidentielle + fuites), les consommations industrielles, commerciales et institutionnelles (ICI) ont généralement un modèle constant vu qu'on n'a pas leurs courbes de modulation ;
4. La consommation des gros ICI provient soit de mesures par compteurs ou d'estimations à l'aide de ratios ;
5. Les entrées sont modélisées comme des réservoirs avec courbe de variation sur 24h des HGL (approche valable pour les scénarios de demande normale). Parfois, c'est le débit qui est imposé à l'entrée. Aux sorties, le débit sortant est imposé comme une demande ;
6. À la construction du modèle hydraulique, des coefficients d'Hazen-Williams (CHW) dits théoriques sont affectés provisoirement aux conduites. Pour la fonte grise, ils sont dégradés pour tenir compte de l'âge des conduites. Pour la fonte ductile, installée après 1979, la fourchette par diamètre n'est pas assez large (exemple 8 po : 70 à 90). Pour le PVC- PE : 130 à 150. Pour le béton armé : 100 à 130, etc. À noter que pour la fonte grise à Montréal, les CHW

mesurés sur le terrain sont la plupart du temps proche de ceux qu'on trouve dans la littérature avec « attaque appréciable ou sévère (M32 de l'AWWA) » mais c'est différent d'un secteur à un autre et aussi de la période d'installation. La période de 1940-1965 est connue par la mauvaise qualité de la fonte avec plusieurs bris et corrosion accélérée ;

7. Mesure des CHW sur terrain :

   7.1. Étude d'échantillonnage des conduites du secteur étudié en fonction des matériaux, âges et emplacements. Conduites ciblées en fonte grise et fonte ductile avant 1979 (non revêtues), parfois quelques conduites 8 po fonte ductile des années 80 ;

   7.2. À noter que deux conduites fonte grise de mêmes âges peuvent avoir des CHW mesurés différents ;

8. Validation par des tests débits/pressions aux bornes (on peut aussi l'appeler calibration par les tests débit/pression) :

   8.1. Macro calibration par le module « Darwin Calibrator » : création de groupes de conduites en fonction de l'âge, matériaux et emplacement. Le but est de modifier globalement les CHW tout en s'inspirant des CHW mesurés et théoriques, pour rapprocher les pressions statiques avant écoulement ;

   8.2. Calibration manuelle : modifier les CHW des conduites locales autour de la borne pour rapprocher les pressions résiduelles terrain-modèle. Ne pas affecter des CHW qui n'ont pas de sens, il faut chercher l'origine des écarts. Parfois il s'agit des vannes fermées, partiellement ouvertes, de la consommation, des fuites, de la topologie, des erreurs dans le modèle... ;

   8.3. Commercer la calibration par les tests en allant de la source vers l'aval ou le centre du secteur s'il y a des entrées autour du dit secteur ;

   8.4. Implanter les tests au long des conduites majeures de distribution 10 po à 16 po en allant de la source vers l'aval, une bonne calibration de ces conduites sera bénéfique pour l'ensemble des tests ;

   8.5. Généralement un écart maximal de 5 psi pourrait être acceptable en terme de pression résiduelle, mais il faut en tenir compte de cette erreur lors de l'évaluation de la capacité hydraulique du réseau et des pressions statiques ;

Tout le travail fait à ce stade sert à utiliser le modèle hydraulique en vue de faire une proposition d'un SSD temporaire, des ajustements sont faits par la suite.

9. Implantation temporaire du secteur visé :

    9.1. Campagne de mesure des pressions et demande en eau du secteur visé pendant l'été aux entrées (on cible la demande en eau maximale) ;

    9.2. Campagne de mesure des pressions statiques aux bornes à l'intérieur du secteur ;

    9.3. Essais écoulement aux bornes incendie (points critiques et quelques points moyens) : même méthodologie que débits/pressions mais seulement avec une borne témoin. Le but de ces essais est de :

        9.3.1. Évaluer sur terrain l'impact de la sectorisation sur les débits soutirés aux bornes et les pressions résiduelles aux bornes témoins (critère : moins de 20% de perte de débit et moins de 10% en moyenne pour l'ensemble des bornes étudiées) ;

        9.3.2. Ajuster le modèle hydraulique si requis (une sorte de validation puisqu'il s'agit de conditions hydrauliques différentes) : ici la plupart du temps on fait quelques modifications des CHW pour ajuster les pressions résiduelles et les pressions statiques sur 24h (les écarts en statique sont généralement très faibles ce qui est normal).

APPENDIX 2 – SUPPLEMENTAL INFORMATION, ARTICLE 1: PREDICTING WATER QUALITY IMPACT AFTER DMAS IMPLEMENTATION IN A FULL-SCALE DWDS

Journal: Journal AWWA

Title: Predicting water quality impact after DMAs implementation in a full-scale DWDS

Authors: Vanessa C. F. Dias, Michèle Prévost, Marie-Claude Besner

Number of pages: 5

Number of figures: 4

Figure A-2.1

Figure A-2.2

Figure A-2.3

Figure A-2.4

Number of tables: 1

Table A-2.1

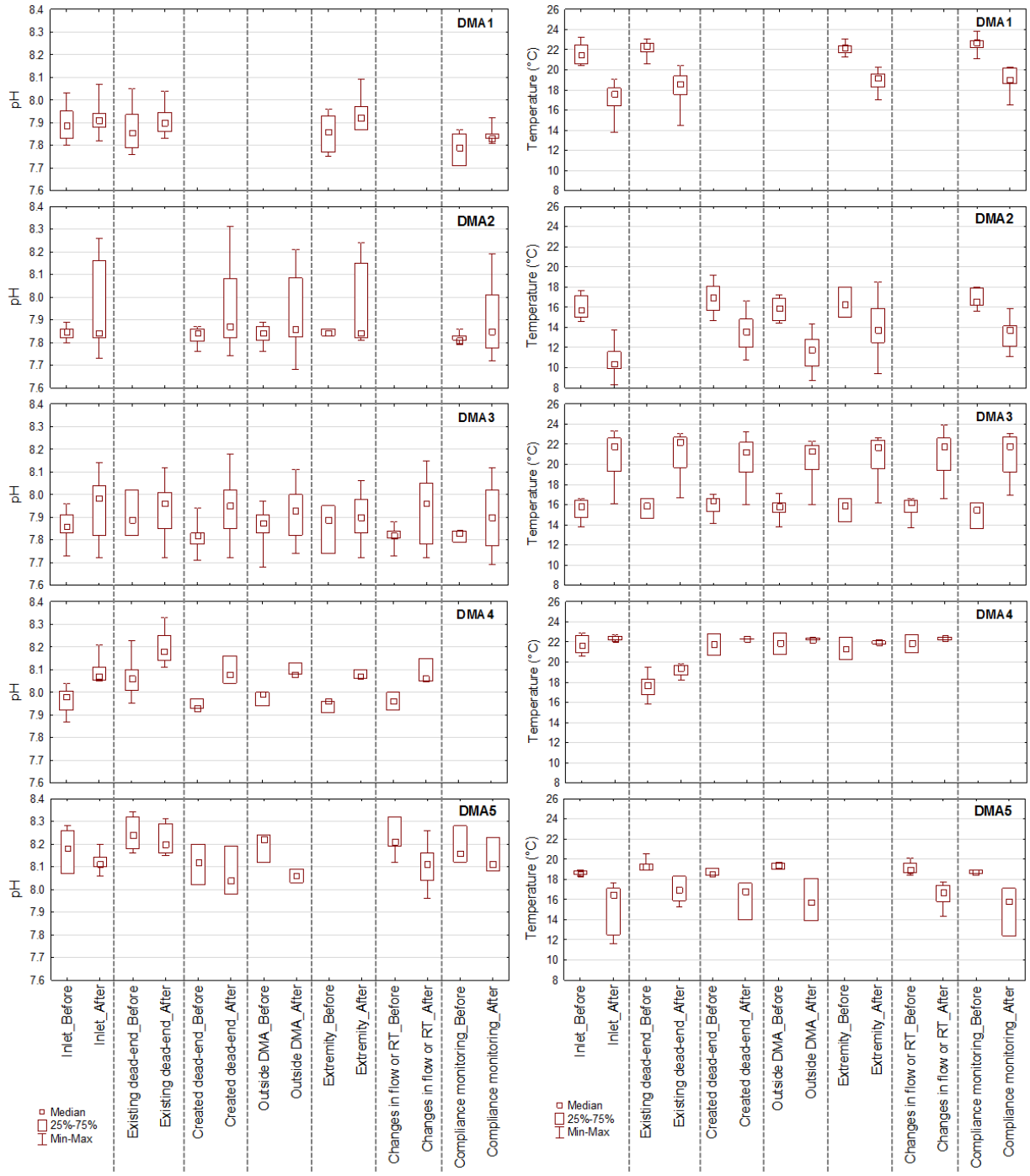

Figure A-2.1: Box-and-whisker plots of pH and temperature across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values

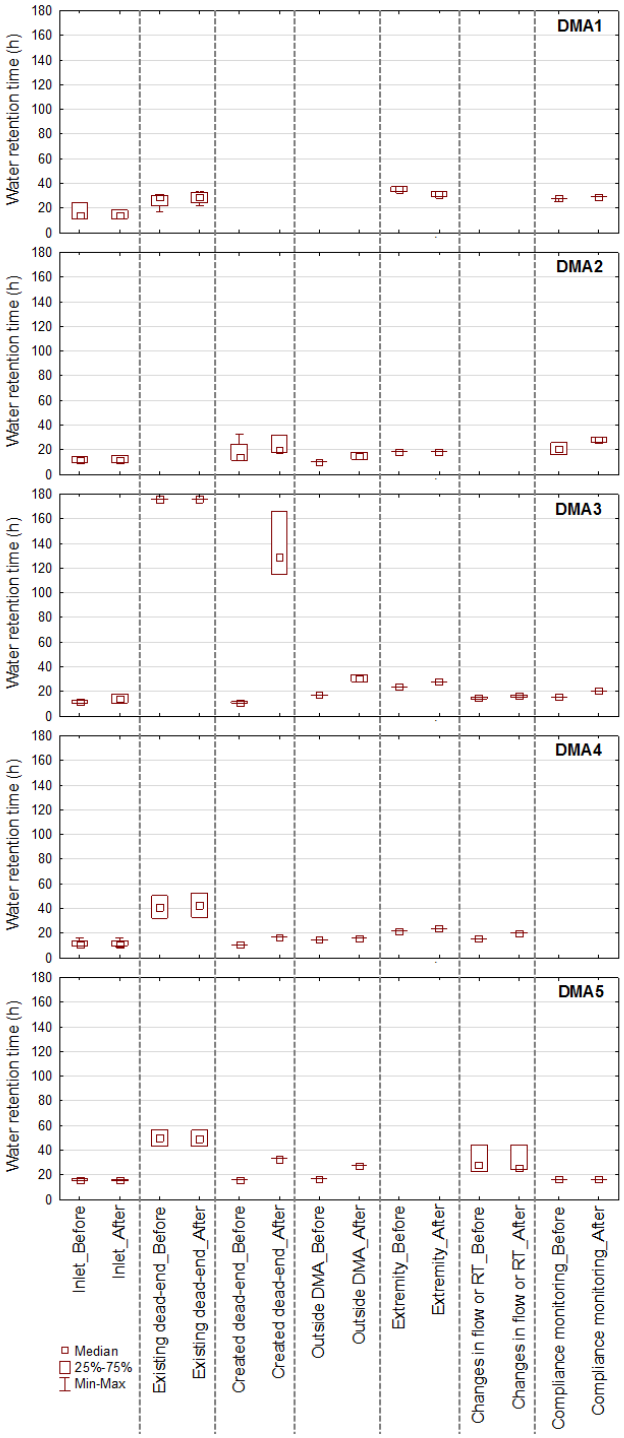

Figure A-2.2: Box-and-whisker plots of water residence time across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values

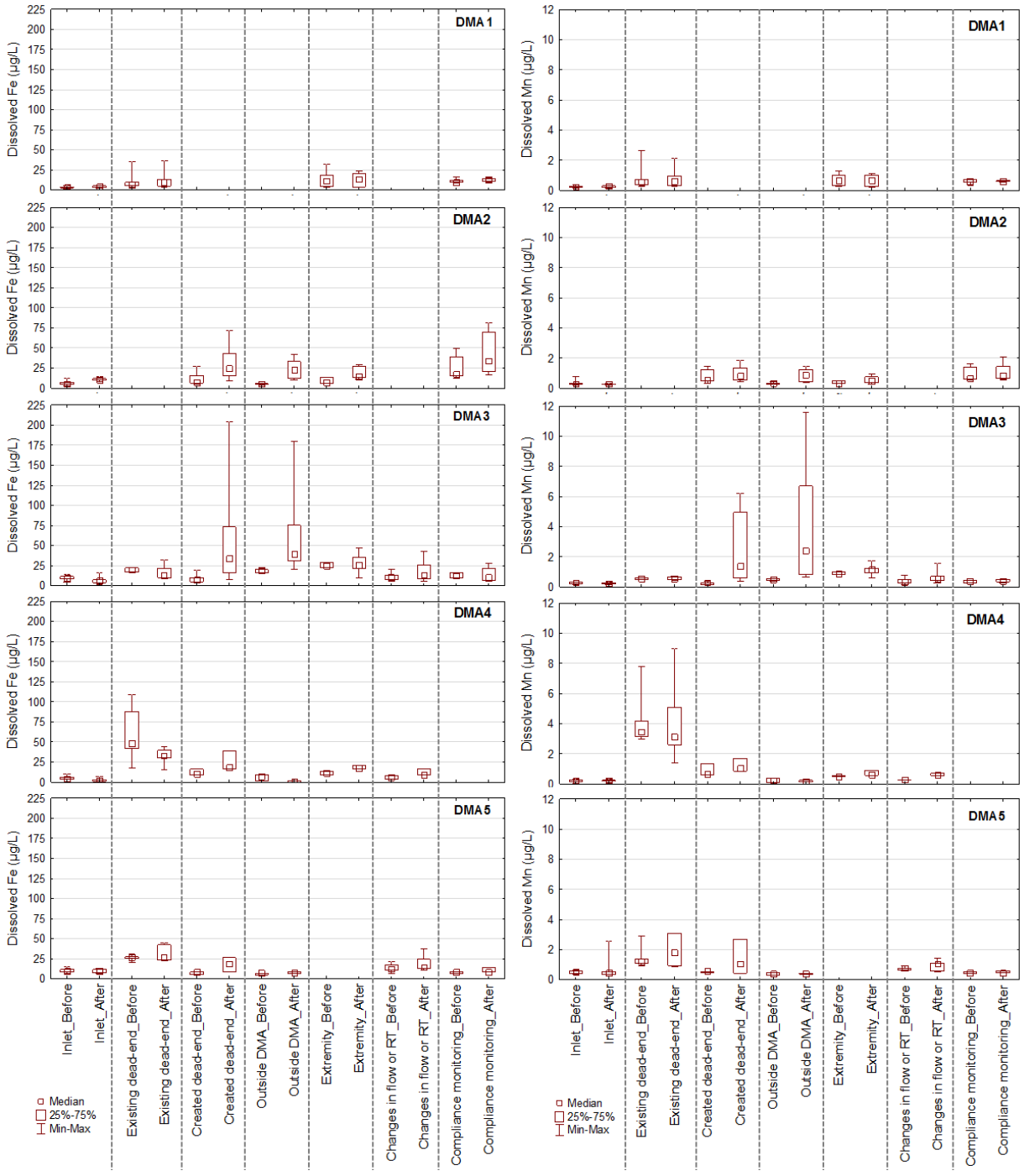

Figure A-2.3: Box-and-whisker plots of dissolved iron (Fe) and manganese (Mn) across all sampling locations in each DMA. Boxes represent 25th and 75th quartiles of values, whiskers minimum and maximum values and the black squares median values

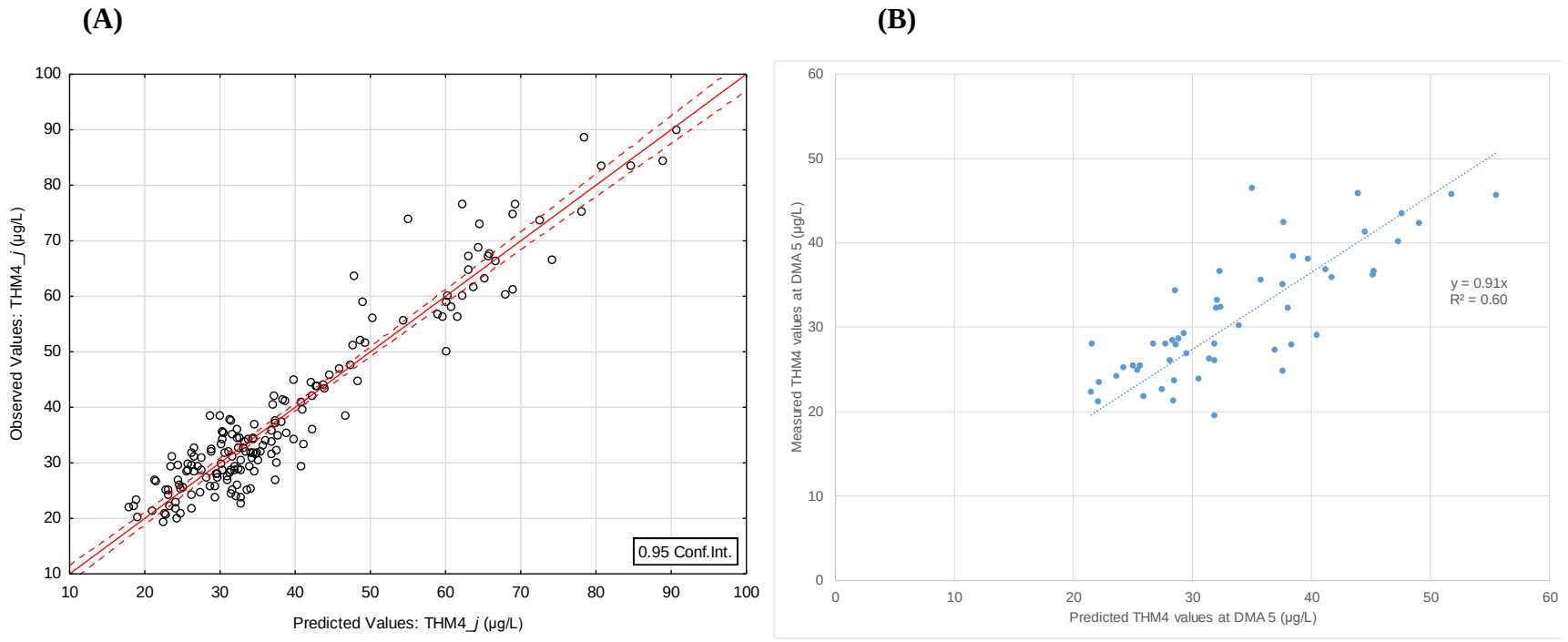

Figure A-2.4: Predicted THM4_i values vs. observed values from the regression model (A) and validation of the regression model at DMA 5 using the regression model from DMAs 1-4 (B)

Table A-2.1: Pearson's correlations between measured water quality parameters in DMAs 1-5 (red correlations are significant at $\rho<0.05$)

Variable	**pH (n=525)**	**Temperature (n=525)**	**Free chlorine (n=525)**	**Turbidity (n=460)**	**DOC (n=372)**	**THM4 (n=460)**	**HAA6 (n=361)**	**Dissolved Fe (n=460)**	**Total Fe (n=460)**	**Dissolved Mn (n=460)**	**Total Mn (n=460)**	**WRT (n=525)**
pH	1.00	-0.03	-0.18	0.02	-0.10	-0.17	-0.30	-0.07	0.09	0.07	0.10	0.01
Temperature	-0.03	1.00	-0.31	-0.02	-0.41	0.01	-0.06	-0.16	-0.03	0.02	0.00	0.17
Free Cl_2	-0.18	-0.31	1.00	-0.39	0.02	-0.38	-0.01	-0.50	-0.54	-0.53	-0.56	-0.45
Turbidity	0.02	-0.02	-0.39	1.00	0.11	0.32	0.07	0.49	0.79	0.53	0.73	0.19
DOC	-0.10	-0.41	0.02	0.11	1.00	0.73	0.73	0.34	0.18	0.08	0.09	0.17
THM4	-0.17	0.01	-0.38	0.32	0.73	1.00	0.76	0.52	0.41	0.37	0.32	0.41
HAA6	-0.30	-0.06	-0.01	0.07	0.73	0.76	1.00	0.26	0.13	0.05	0.01	0.20
Dis. Fe	-0.07	-0.16	-0.50	0.49	0.34	0.52	0.26	1.00	0.77	0.79	0.73	0.32
Total Fe	0.09	-0.03	-0.54	0.79	0.18	0.41	0.13	0.77	1.00	0.75	0.79	0.32
Dis. Mn	0.07	0.02	-0.53	0.53	0.08	0.37	0.05	0.79	0.75	1.00	0.85	0.23
Total Mn	0.10	0.00	-0.56	0.73	0.09	0.32	0.01	0.73	0.79	0.85	1.00	0.22
WRT	0.01	0.17	-0.45	0.19	0.17	0.41	0.20	0.32	0.32	0.23	0.22	1.00

APPENDIX 3 – SUPPLEMENTAL INFORMATION: FORMATION OF THMS AT CHLORINATED TREATMENT PLANT WATER

Title: Informations sur la demande en chlore et la formation des THM – mémorandum envoyé à la ville de Montréal le 3 septembre 2013

Authors: Michèle Prévost, Vanessa C. F. Dias

Number of pages: 6

Number of figures: 4

Informations sur la demande en chlore et la formation des THM

Pour vous aider à réfléchir sur votre stratégie de chloration en usine et en réseau, voici des résultats d'essais au laboratoire qui fournissent des informations sur la cinétique de consommation du chlore et de formation de THM. Vanessa doit encore bien exploiter ces résultats en les modélisant mais nous croyons qu'ils vous aideront dans votre réflexion.

La situation actuelle dans les réseaux desservis par les usines DesBaillets et Atwater est temporaire puisqu'il s'agit d'une période de transition en raison des travaux en cours dans les usines, particulièrement le changement de type de chloration. Une fois l'ozonation et les UV en route, il sera possible de réévaluer les besoins de chloration en fonction d'objectifs de maintien d'un résiduel en réseau et de limitation de la formation des THM. Typiquement, on retient une proportion fixe de la norme de THM comme objectif d'exploitation. Par exemple, aux États-Unis, la norme de THM est de 80ug/L et les exploitants doivent développer un plan de réduction des THM lorsque 80% de ce niveau est atteint, soit 64ug/L. Dans le cas du résiduel, il faut poser la question du besoin de maintien de concentrations résiduelles de chlore à tous les points du réseau, particulièrement dans les culs-de-sac. Plus un réseau est en bon état, moins il est souhaitable de maintenir un résiduel de chlore. Dans le cas du réseau de Montréal, l'état du réseau et le grand nombre d'interventions en cours avec les travaux de réhabilitation justifient certainement le maintien d'un résiduel comme barrière de protection.

On peut identifier plusieurs façons d'atteindre les deux objectifs contradictoires de maintien de résiduel et de limitation des sous-produits d'oxydation. La plus directe est de diminuer les doses de chlore appliquées en usine et par le fait même le résiduel en sortie d'usine, et d'avoir recours à la rechloration en réseau. Il est aussi possible de diminuer les précurseurs de sous-produits en amont de la chloration, ce qui sera une option après la mise en route des nouveaux procédés. Les études précédentes de la chaire ont montré que le démarrage de la pré-ozonation apportera une diminution du potentiel de formation des THM à long terme de 10-30%. La coagulation diminuera aussi le potentiel de formation en fonction de la dose de coagulant appliquée et cette diminution pourrait atteindre 10-15%. Toutefois, en attendant le démarrage de ces procédés la chloration est la seule barrière de désinfection et le maintien des CT est essentiel à la production d'une eau correctement désinfectée.

En préparation des études de suivi de sectorisation, Vanessa a complété en 2012 des essais de formation des THM en fonction de la consommation de chlore sur les eaux filtrées des usines DesBaillets et Atwater. Comme nous anticipions que les études de sectorisation seraient réalisées pendant la période de mise en place de la chloration aux hypochlorites *in situ*, nous voulions comprendre l'impact de la mise en route progressive des deux installations sur la dynamique du chlore et des THM en réseau. Sans ces informations, il serait difficile de comparer les résultats avant et après sectorisation.

Les questions soulevées alors étaient :

- Le type de chlore influence-t-il la demande en chlore ? La stabilité du résiduel est-elle modifiée ?
- Les hypochlorites augmentent le pH et la formation des THM est favorisée par une augmentation de pH au détriment de la formation des AHA. L'augmentation de pH associée à l'utilisation des hypochlorites augmente-t-elle la formation de THM ?
- Les légères variations de COT sont-elles associées à des augmentations significatives de demande en chlore et de THM ?

Les essais ont été réalisés en laboratoire avec de l'eau prélevée en décembre 2011, février et avril 2012. Pendant cette période, la concentration en matières organiques de l'eau a varié légèrement (COT de 2,2 à 2,6 mg/L). Les échantillons d'eau filtrée prélevés aux deux usines ont été chlorés avec de l'hypochlorite et comparés à des prélèvements d'eau chlorée au chlore gazeux en usine. Les essais ont été effectués à 5^{o}C et 20^{o}C pour préciser l'impact de la température. De plus, le pH a été contrôlé par l'ajout d'un tampon phosphate pour bien distinguer l'impact du pH. Les doses d'hypochlorites utilisées ont varié de 1,5 à 4 mg/L pour demeurer dans une gamme de valeurs réalistes.

La Figure A-3.1 montre un exemple des résultats de demande en chlore en fonction du temps d'incubation au laboratoire à 5 et 20^{o}C. Le trait en pointillé rouge montre la décroissance du chlore dans l'échantillon d'eau filtrée chlorée en usine et incubée au laboratoire. Cette référence permet une comparaison entre la décroissance du chlore appliqué à l'usine à un pH d'environ 7,9 à celle de l'eau filtrée traitée avec des hypochlorites à pH tamponné à 7,8, et à pH variant selon la dose d'hypochlorites de sodium. On remarque une décroissance légèrement plus rapide de l'échantillon

chloré à l'usine à 20ºC, quel que soit le pH de l'eau après ajout d'hypochlorites. En eau froide, la décroissance du chlore des échantillons avec hypochlorites est légèrement plus rapide à pH de 7,8 qu'à pH ambiant (8-8,2). La différence la plus notable est que la demande et la cinétique de décroissance de chlore augmentent considérablement lorsque la dose d'hypochlorites est augmentée à 3 mg/L.

La Figure A-3.2 montre un exemple de formation de THM pour différentes conditions de chloration. On voit à la fois l'effet de la dose de chlore et de la température. La Figure A-3.3 montre l'ensemble des résultats de formation de THM à différentes doses de chlore en fonction de la consommation de chlore. Les résultats sont séparés de manière à visualiser facilement les résultats de l'eau chlorée avec du chlore gazeux en usine (en carrés rouges), des hypochlorites de sodium à pH variant avec la dose appliquée de 8 à 8,5, et des hypochlorites dans l'eau tamponnée à pH 7,8.

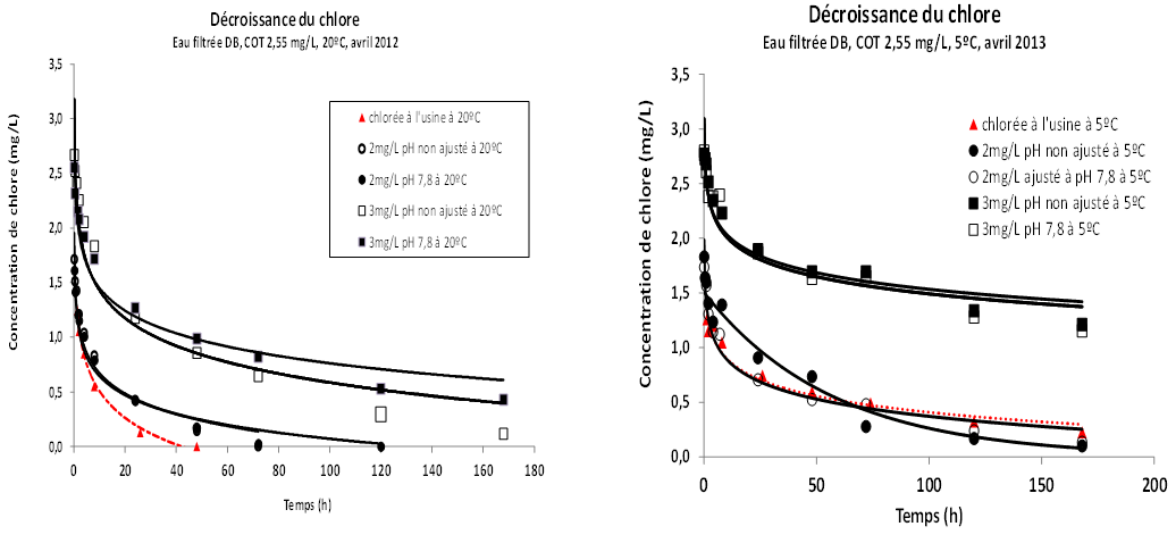

Figure A-3.1: Décroissance du chlore dans l'eau filtrée de l'usine DesBaillets en avril 2012 à 5° et 20°C

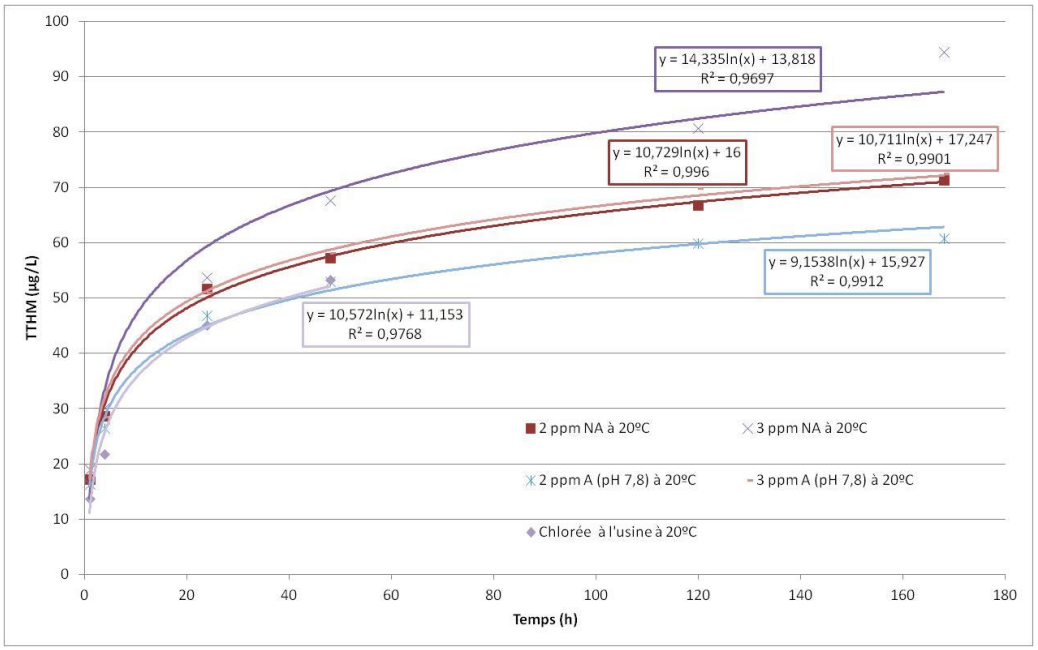

Figure A-3.2: Cinétique de formation de THM en fonction de la dose de chlore (2 et 3 mg/L avec et sans ajustement de pH) et de la température (5° et 20°C)

Les régressions d'excellente qualité (R^2>0,86) montrent le potentiel de formation de THM exprimé en mg THM/mg Cl_2 consommé. Les pentes de ces régressions montrent des formations comparables (31,0 à 33,0 mg THM/mg Cl_2) pour toutes les conditions testées. Une formation très légèrement supérieure est observée à pH ambiant (8-8,5) après l'ajout d'hypochlorites. On pourrait se demander si les légères variations de COT peuvent expliquer la variabilité des données. Une vérification des régressions pour chaque valeur de COT a révélé que la réactivité de la matière organique varie dans le temps et qu'une concentration de COT plus élevée (2,6mg/L) ne forme pas forcément plus de THM qu'un COT légèrement plus faible (2,2mg/L).

Ces valeurs de formation à dosages de Cl_2 réalistes montrent un potentiel de formation relativement modeste en comparaison avec ceux observés pour d'autres eaux de surface. Par exemple, Vaillancourt a observé des formations de 100 à 150 mg THM/mg Cl_2 sur quatre eaux de surface. La formation des THM est influencée par de nombreux paramètres dont la teneur en matières organique (COD), la dose de chlore, le pH et la température. L'influence relative de ces paramètres a été étudiée par de nombreux auteurs et est particulièrement bien illustrée par l'application du modèle d'Amy et collaborateurs (dans Vaillancourt, 2006).

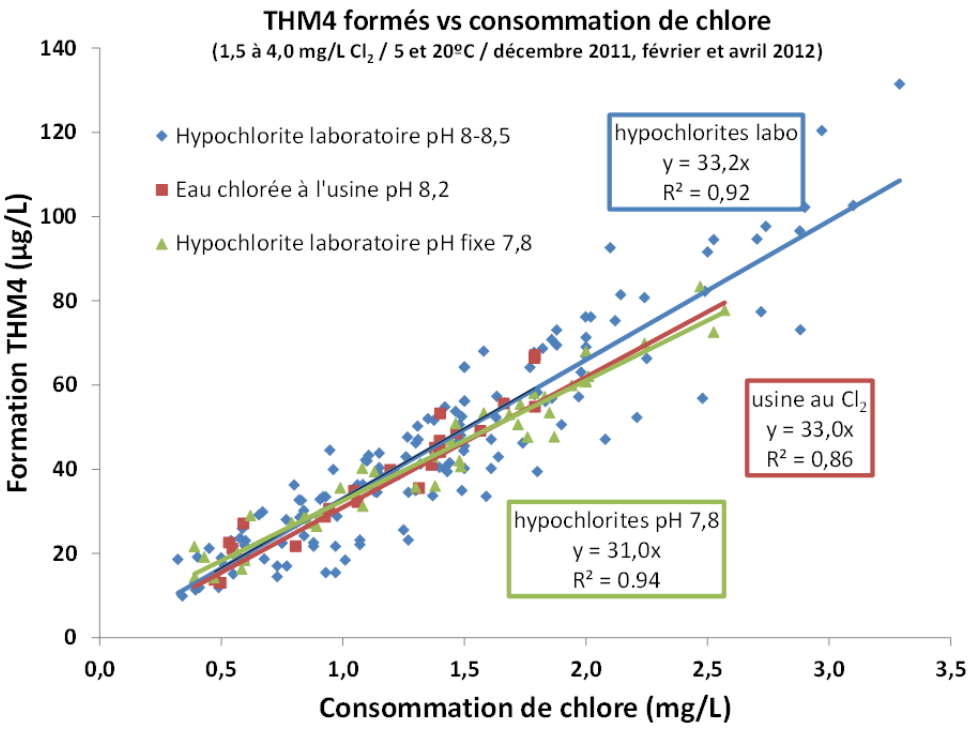

Figure A-3.3: Formation de THM4 en fonction de la consommation de chlore pour des eaux filtrées des usines Atwater et DesBaillets. Températures d'incubation de 5° et 20°C avec ajout d'hypochlorites variant de 1,5 à 4 mg/L et des pH ambiants ou fixes à 7,8

L'évolution saisonnière des concentrations en matières organiques est clairement illustrée à la Figure A.3-4 pour l'eau filtrée de l'usine DesBaillets. On note des augmentations vers le mois d'avril chaque année jusqu'à 3,8 mg/L COT. Les essais présentés ici ne sont pas représentatifs de résultats avec les COT très élevés observés au printemps qui peuvent atteindre 3,8 mg/L. Il est tout à fait probable qu'une demande en chlore additionnelle soit associée à cette augmentation printanière et que les concentrations de THM formés à ce moment soient aussi augmentées. L'augmentation de COT est probablement attribuable à la plus grande proportion des eaux de l'Outaouais dans l'eau brute pendant cette période de l'année. Toutefois, l'augmentation du potentiel de formation de THM pourrait être contrôlée par une coagulation.

À noter que l'abaissement de la température cause un ralentissement de l'ensemble des cinétiques de réaction avec la matière organique qui se traduit par une demande en chlore plus lente résultant en une formation réduite de THM. C'est pourquoi l'influence de la température n'est pas visible à

la Figure 1 qui regroupe pourtant des valeurs obtenues à 5º et 20ºC. Les variations de COT et de pH durant ces essais sont modestes et leur influence limitée. Par contre, le dosage de chlore est un facteur majeur qui influence à la fois la cinétique de formation et la valeur maximale de THM formés, particulièrement lorsque des doses typiques de celles utilisées en exploitation sont appliquées.

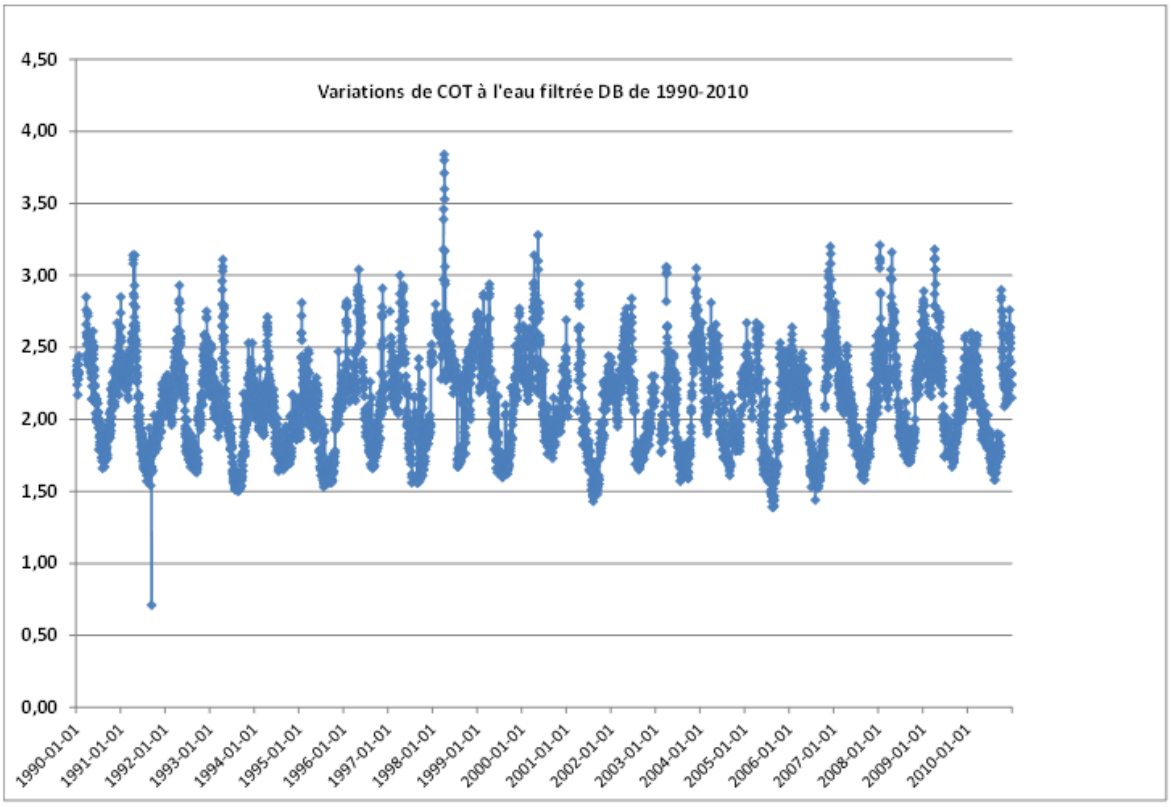

Figure A-3.4: Évolution du COT à l'eau filtrée de l'usine DesBaillets, 1990-2010

Références

Vaillancourt, F. (2006). Guide de vérification du respect des normes sur les sous-produits de désinfection à l'intention des installations ne désirant pas filtrer leurs eaux (Maîtrise). Génies Civil, Géologique et des Mines, École Polytechnique de Montréal, Québec, Canada, p.165.

APPENDIX 4 – SUPPLEMENTAL INFORMATION, ARTICLE 2: ASSESSING THE IMPACT OF DMA IMPLEMENTATION ON BACTERIAL WATER QUALITY IN A FULL-SCALE DS

Journal: Journal Plos One

Title: Assessing the impact of DMA implementation on bacterial water quality in a full-scale DS

Authors: Vanessa C. F. Dias, Michèle Prévost, Emilie Bédard, Audrey-Anne Durand, Philippe Constant, Eric Déziel

Number of pages: 4

Number of figures: 2

Figure A-4.1

Figure A-4.2

Number of tables: 2

Table A-4.1

Table A-4.2

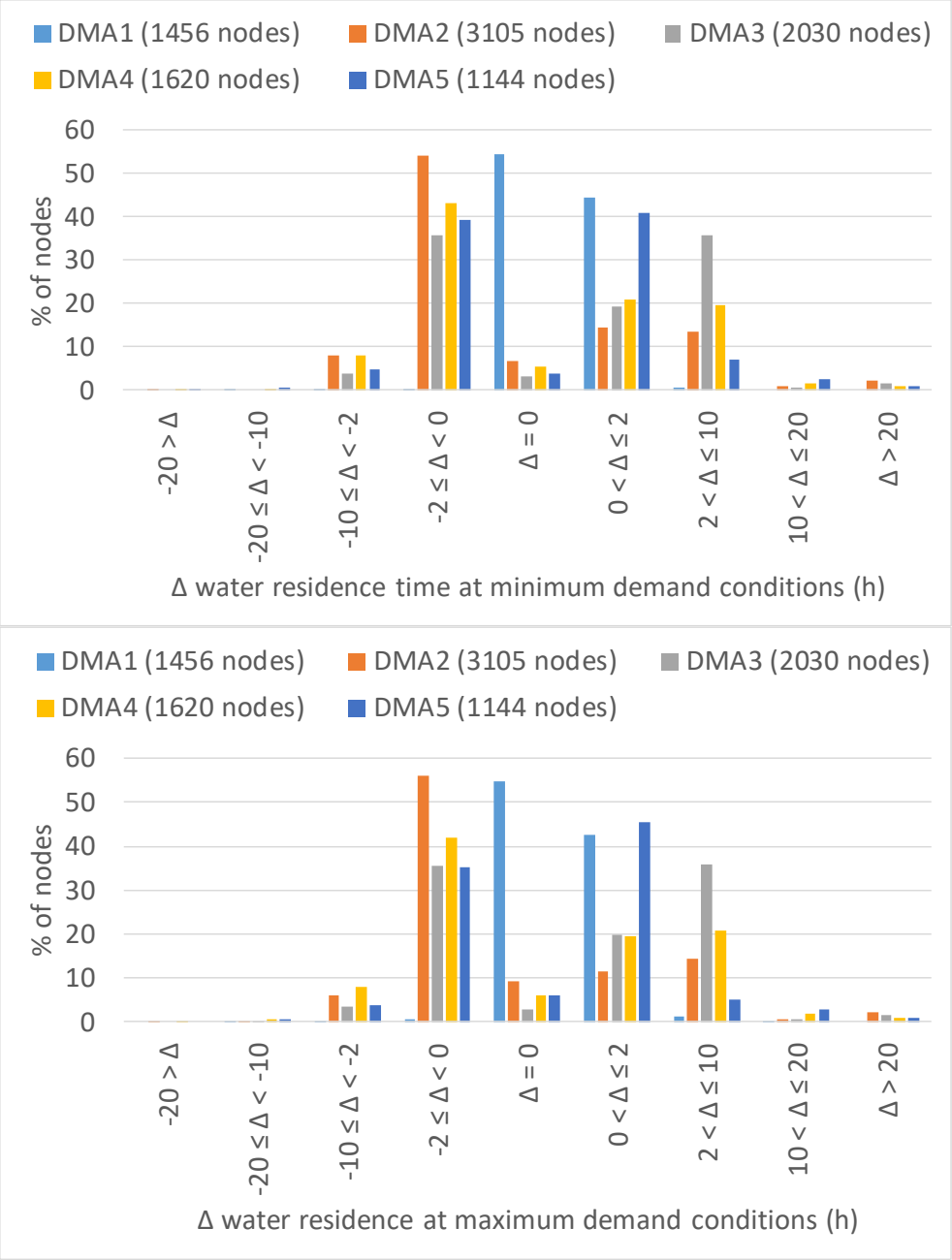

Figure A-4.1: Variations in water residence time at minimum demand conditions and maximum demand conditions

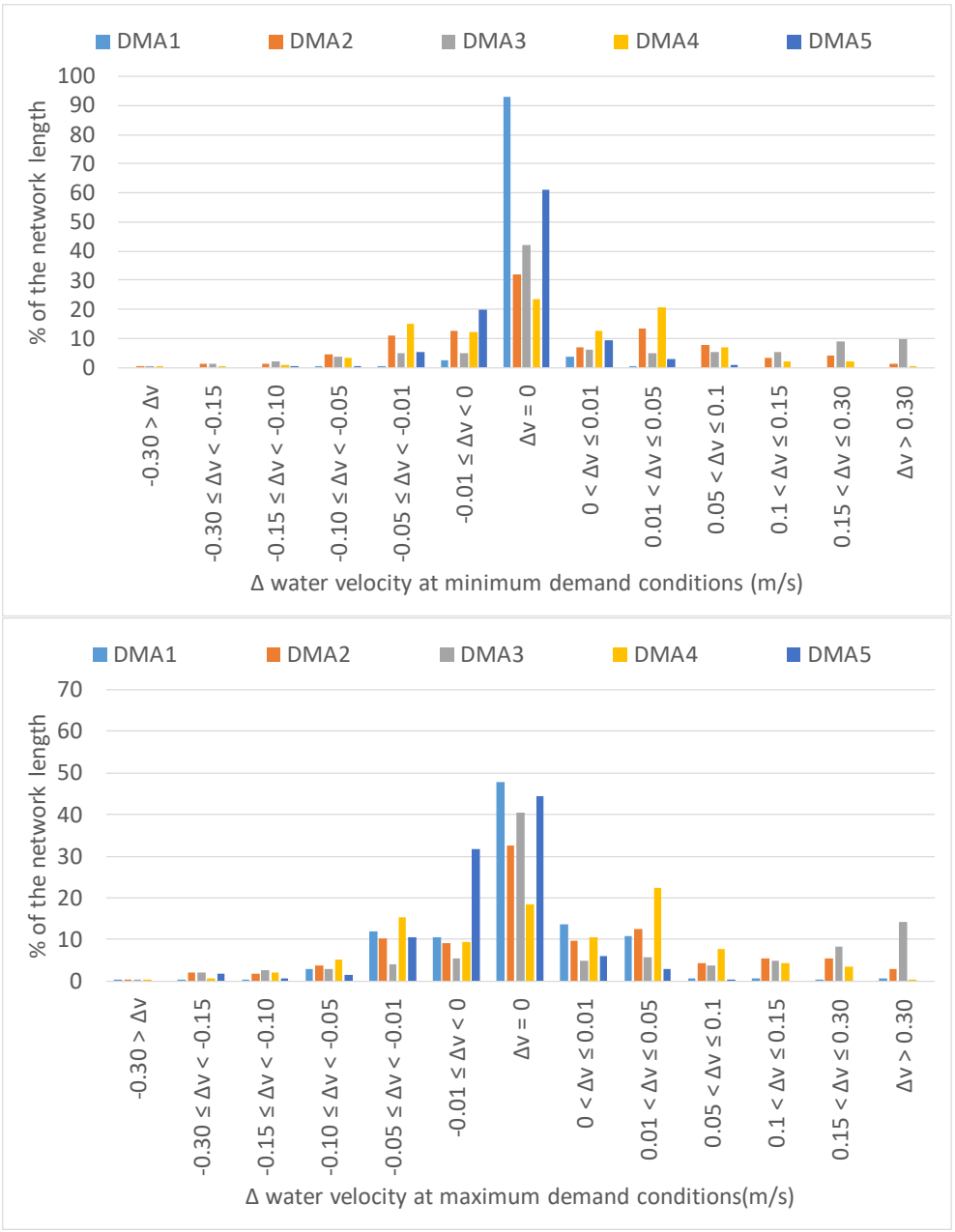

Figure A-4.2: Variations in water velocity at minimum demand conditions and maximum demand conditions

Table A-4.1: Pearson's correlations between measured water quality parameters and log HPC for each DMA and for all DMAs combined (red correlations are significant at $\rho<0.05$)

Parameter	DMA1		DMA2		DMA3		DMA4		DMA5		DMAs 1-5	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
pH	100	0.13	106	0.09	147	0.10	60	0.54	60	0.14	473	0.22
Temperature	100	0.28	106	0.11	147	0.09	60	-0.59	60	0.21	473	0.16
Free chlorine	100	-0.49	106	-0.17	147	-0.73	60	-0.83	60	-0.61	473	-0.64
Turbidity	100	0.28	106	0.00	133	0.47	60	0.46	60	0.41	459	0.31
Particle counts	100	0.18	106	0.10	133	0.13	60	0.07	60	0.11	459	0.04
DOC	100	-0.05	106	-0.08	86	-0.07	40	-0.43	40	-0.43	372	-0.01
Dissolved Fe	100	0.39	106	0.12	133	0.52	60	0.66	60	0.45	459	0.37
Total Fe	100	0.38	106	0.17	133	0.56	60	0.69	60	0.47	459	0.44
Dissolved Mn	100	0.39	106	0.23	133	0.54	60	0.67	60	0.44	459	0.46
Total Mn	100	0.45	106	0.04	133	0.55	60	0.67	60	0.22	459	0.42
Total cell counts	100	0.22	106	-0.07	51	0.16	30	0.82	20	0.56	307	0.32
Residence time	100	0.46	106	0.00	112	0.56*	60	0.76	60	0.49	43	0.51*

*Dead-ends with water residence times between 115 hours and 176 hours were excluded from these analyses (4 dead-ends from DMA3: 1 existing and 3 created ones).

Table A-4.2: Pearson's correlations between measured water quality parameters and log total cell counts for each DMA and for all DMAs combined (red correlations are significant at $\rho<0.05$)

Parameter	DMA1		DMA2		DMA3		DMA4		DMA5		DMAs 1-5	
	n	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*	*n*	*r*
pH	100	0.15	106	-0.14	52	0.22	30	0.58	20	0.68	308	-0.08
Temperature	100	0.71	106	0.43	52	-0.60	30	-0.68	20	0.57	308	0.25
Free chlorine	100	-0.31	106	-0.22	52	-0.10	30	-0.89	20	-0.81	308	-0.44
Turbidity	100	0.29	106	0.08	52	-0.12	30	0.55	20	0.47	308	0.09
Particle counts	100	0.44	106	0.05	52	-0.16	30	0.05	20	0.10	308	0.17
DOC	100	-0.55	106	-0.27	39	0.09	30	-0.36	20	-0.73	295	-0.11
Dissolved Fe	100	0.07	106	0.06	52	-0.05	30	0.68	20	0.55	308	0.07
Total Fe	100	0.19	106	0.07	52	-0.03	30	0.77	20	0.64	308	0.15
Dissolved Mn	100	0.11	106	0.12	51	0.02	30	0.79	20	0.64	307	0.19
Total Mn	100	0.29	106	0.08	52	0.01	30	0.78	20	0.25	308	0.15
HPC	100	0.22	106	-0.07	51	0.16	30	0.82	20	0.56	307	0.32
Residence time	100	0.12	106	0.18	42	-0.12*	30	0.84	20	0.62	298	0.32*

*Dead-ends with water residence times between 115 hours and 176 hours were excluded from these analyses (4 dead-ends from DMA3: 1 existing and 3 created ones).

APPENDIX 5 – SUPPLEMENTAL INFORMATION, ARTICLE 3: IDENTIFICATION OF FACTORS AFFECTING BACTERIAL ABUNDANCE AND COMMUNITY STRUCTURES IN A FULL-SCALE DWDS

Journal: Journal Applied and Environmental Microbiology

Title: Identification of factors affecting bacterial abundance and community structures in a full-scale DWDS

Authors: Vanessa C. F. Dias, Emilie Bédard, Audrey-Anne Durand, Philippe Constant, Eric Déziel, Michèle Prévost

Number of pages: 4

Number of figures: 1

Figure A-5.1

Number of tables: 3

Table A-5.1

Table A-5.2

Table A-5.3

Table A-5.1: Characteristics of the samples from premise plumbing

Sample	Type of tap	Floor	Department
TAP1	Standard	3	Intensive care
TAP2	Standard	3	Intensive care
TAP3	Standard	6	Transplantation
TAP4	Standard	6	Transplantation
TAP5	Standard	3	Transplant
TAP6	Pedal	2	Oncology
TAP7	Standard	2	Oncology
TAP8	Standard	3	Transplant
TAP9	Pedal	4	Neonatology
TAP10	Pedal	3	Transplant

Table A-5.2: Average estimators of alpha-diversity for treated water (TW), distributed water (DS) and tap water (TAP)

Sub-system	Coverage[a]	Shannon	Ace	Chao	Evenness[b]
TW	0.98 ± 0.01	3.21 ± 0.26	105 ± 28	106 ± 28	0.72 ± 0.03
DS	0.97 ± 0.01	2.21 ± 1.18	138 ± 51	108 ± 30	0.51 ± 0.23
TAP	0.97 ± 0.01	2.64 ± 0.91	115 ± 50	101 ± 54	0.62 ± 0.14

a. Good's coverage.

b. Shannon index-based measure of evenness.

Table A-5.3: Frequency of detection of genera containing OPs across the sub-systems

Genera	TW	DS	TAP
Legionella spp.	0/7	0/10	3/10
Mycobacterium spp.	7/7	0/10	1/10
Pseudomonas spp.	4/7	8/10	2/10
Sphingomonas spp.	4/7	9/10	10/10

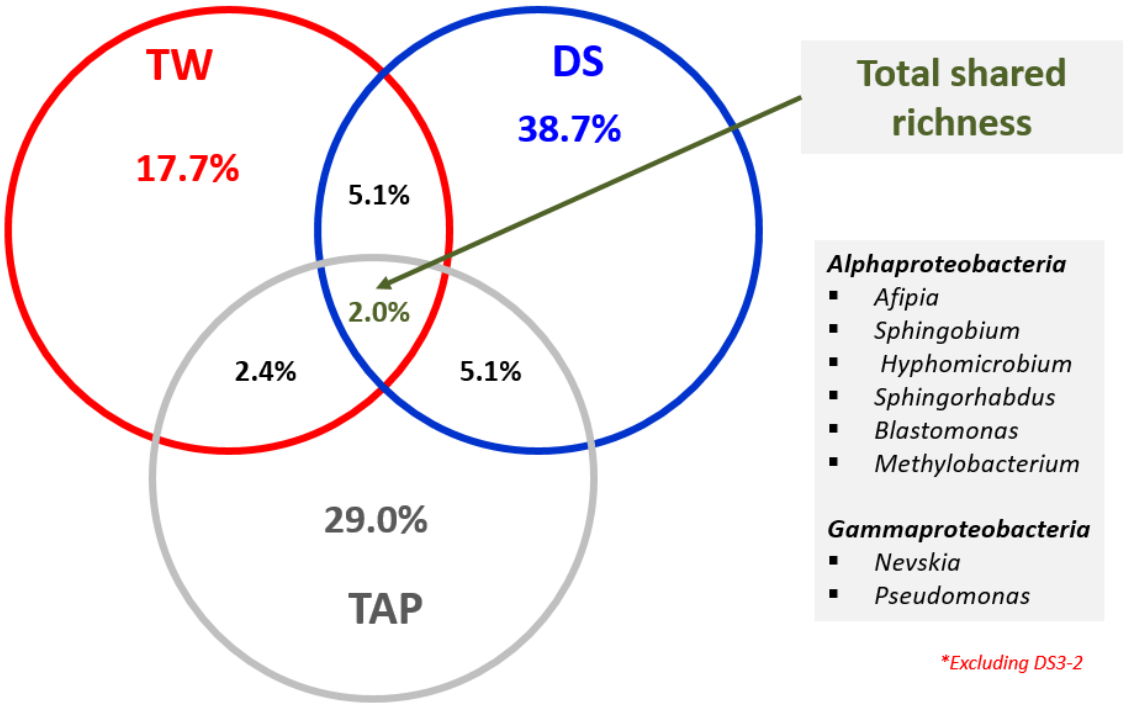

Figure A-5.1: Venn diagram showing the shared OTUs across the sub-systems